National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

NL-339 (r. 88/04)

Canada

THE UNIVERSITY OF ALBERTA

EVALUATION OF NUTRITIONAL QUALITY OF PROTEIN SOURCES FOR CATTLE

by

B.K. KIRKPATRICK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-42834-1

University of Alberta — Inter-departmental Correspondence

to: Audrey Rode
Faculty Graduate Studies & Research

date: 1988 June 24

our file: Ref:K1.2-mem-june24

from: J.J. Kennelly
Department of Animal Science

your file:

subject:

This is to confirm that I grant permission to Bonnie Kirkpatrick to use Chapter II of her thesis which I co-authored. This chapter was published in the Journal of Animal Science.

J.J. Kennelly

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR B.K. KIRKPATRICK

TITLE OF THESIS EVALUATION OF NUTRITIONAL QUALITY OF PROTEIN SOURCES FOR CATTLE

DEGREE FOR WHICH THESIS WAS PRESENTED DOCTOR OF PHILOSOPHY

YEAR THIS DEGREE GRANTED SPRING 1988

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) B.K. Kirkpatrick

PERMANENT ADDRESS:

10926 Capilano Rd

Edmonton

Alberta

DATED April 25 19 88

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EVALUATION OF NUTRITIONAL QUALITY OF PROTEIN SOURCES FOR CATTLE submitted by B.K. KIRKPATRICK in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL NUTRITION.

.....

Supervisor

.....

.....

.....

.....

External Examiner

Date April 25/1988

ABSTRACT

To evaluate the quality of protein sources for dairy cattle, estimates of rumen degradable protein (RDP) and rumen undegradable protein (UDP) were determined by both in situ, Experiment I, and in vivo, Experiment II, techniques. A method for evaluating protein availability in the small intestine - the mobile nylon bag technique (MNBT) - was developed Experiment III. Total diet dry matter digestibility (DMD) and crude protein digestibility (CPD) were determined using the traditional total fecal collection technique (TFC), the MNBT and the rare earth marker, dysprosium (Dy), Experiment IV. Six diets were fed 12 times daily to six pregnant Holstein heifers with rumen and duodenal cannulae in a 6x6 Latin square design. The protein sources evaluated were barley (B), canola meal (CM), soybean meal (SBM) and meat and bone meal (MBM). The six diets (50:50 hay/concentrate (DM basis)) were formulated to provide different levels of CP in the ration: B, 14% CP; CM, 16.5 and 19% CP; SBM, 16.5 and 19% CP; MBM, 16.5% CP. The MBM16.5, CM19 and SBM19 were equivalent on a UDP basis. Estimated values of UDP values for the MBM diet were highest and ranged from 35.0% as determined by the in situ technique to 40.6% by the in vivo technique. However, UDP values for the SBM diets increased with increasing protein levels using the in situ technique. The reverse was true for the in vivo technique. Results for the control and CM diets were similar with both techniques. In conclusion the formulation of diets to increase the UDP level was effective in increasing the amount of feed N reaching the small intestine suggesting that animal performance can be more accurately predicted when diets are formulated on the basis of RDP and UDP. To overcome some of the difficulty and expense involved in conducting conventional digestibility studies in large ruminants, the MNBT was developed and the Dy - ratio technique was evaluated. The MNBT was used successfully to determine intestinal and total tract digestibility of both individual protein sources and total diets. Crude protein digestibilities using the MNBT and the TFC technique for the six complete diets were: 65.6, 67.2; 70.8, 73.0; 70.0, 72.8; 71.7, 72.9; 73.6, 75.8; 77.0, 78.4; respectively. When Dy was used as a digestibility marker, ingested Dy was recovered at

the rate of 95.1% and there was no evidence of daily variation following a 7 day adjustment period. Dry matter and CP digestibilities using the TFC technique were highly correlated to results using the Dy ratio technique ($r = .86$; and .75 for DM and CP, respectively).

ACKNOWLEDGEMENTS

The experiments described herein were supported in part by the Farming For The Future Program of Alberta Agriculture.

I wish to thank Mr. Gary Van Doesburg and Mr. Tom Huwiler for their excellent care and management of the animals used in my studies. The assistance of Mr. Jack Francis and Ms. Charlane Shellian during the experimental collection periods is appreciated. I am very grateful to Ms. Debbie Dalton and Ms. Maxine Langman for their technical assistance with the animals and in the lab.

I am truly grateful to Dr. John Kennelly for his constant guidance in my research. His thoughtfulness, enthusiasm and faith in my abilities were a constant inspiration during my five years of study.

The tremendous encouragement, support and assistance of my husband, Mr. Neil R. Kirkpatrick, will forever be remembered.

Table of Contents

Chapter		Page
I.	INTRODUCTION	1
	A. BIBLIOGRAPHY	5
II.	IN SITU DEGRADABILITY OF PROTEIN AND DRY MATTER FROM SINGLE PROTEIN SOURCES AND FROM A TOTAL DIET	8
	A. INTRODUCTION	8
	B. MATERIALS AND METHODS	9
	C. RESULTS AND DISCUSSION	11
	D. GENERAL DISCUSSION	17
	E. BIBLIOGRAPHY	25
III.	INFLUENCE OF SUPPLEMENTAL PROTEIN SOURCE AND PROTEIN CONCENTRATION ON RUMINAL AND INTESTINAL DIGESTION	28
	A. INTRODUCTION	28
	B. MATERIALS AND METHODS	29
	C. RESULTS AND DISCUSSION	31
	D. BIBLIOGRAPHY	44
IV.	THE MOBILE NYLON BAG TECHNIQUE AS A PREDICTOR OF THE NUTRITIVE VALUE OF FEEDSTUFFS FOR DAIRY CATTLE	48
	A. INTRODUCTION	48
	B. MATERIALS AND METHODS	48
	C. RESULTS AND DISCUSSION	51
	D. BIBLIOGRAPHY	61
V.	DYSPROSIUM AS A DIGESTIBILITY MARKER FOR CATTLE	63
	A. INTRODUCTION	63
	B. MATERIALS AND METHODS	64
	C. RESULTS AND DISCUSSION	65
	D. BIBLIOGRAPHY	70
VI.	GENERAL DISCUSSION	72

A. BIBLIOGRAPHY 76

List of Tables

Table		Page
II.1	Formulation and Composition of Experimental Diets (%) Dry Matter Basis.	19
II.2	Dry Matter and Crude Protein Disappearance (%) From Nylon Bags as a Function of Time, When Samples of the Protein Source Were Incubated in the Rumen of Heifers Fed Diets Containing the Test Protein Source.	20
II.3	Dry Matter and Crude Protein Disappearance (%) From Nylon Bags as a Function of Time When Samples of the Total Diet Were Incubated In the Rumen of Heifers Fed Diets Containing the Test Protein Source.	21
II.4	Nonlinear Parameters and Effective Degradability of Dry Matter (EDDM) and Crude Protein (EDCP) When Samples of the Protein Source Were Incubated in the Rumen of Animals Fed Diets Containing in Test Protein.	22
II.5	Nonlinear Parameters and Effective Degradability of Dry Matter (EDDM) and Crude Protein (EDCP) When Samples of the Total Diet (TD) Were Incubated.	23
II.6	Effective Degradability of Crude Protein at Different Fractional Outflow Rates When Samples of Crude Protein Sources or Total Diet were Incubated.	24
III.1	Formulation and Composition of Experimental Diets (%) Dry Matter Basis	38
III.2	Effect of Dietary Protein Source and Concentration on Ruminal Volatile Fatty Acid Concentration and Proportion, Ruminal pH and Ammonia (NH_3) Concentration[a]	39
III.3	Effect of Dietary Protein Concentration and Source of Ruminal, Intestinal and Total Tract Apparent Digestion (%) of Dry Matter (DM)[a]	40
III.4	Effect of Supplemental Protein Concentration and Source on Ruminal, Intestinal and Total Tract Apparent Digestion (%) of Organic Matter (OM)[a]	41
III.5	Effect of Supplemental Protein Concentration and Source on Ruminal, Intestinal and Total Tract Apparent Digestion (%) of Nitrogen (N)[a]	42
III.6	Ruminal Degraded Protein (RDP) %, Ruminal Undegraded Protein (UDP) % Calculated on the Basis of In Situ and In Vivo Data Compared to ARC and NRC Estimates of Requirements	43

Table Page

IV.1 Formulation and Composition of Experimental Diets (% dry matter basis). 55

IV.2 Ruminal and intestinal crude protein disappearance (%) of protein source (PS) samples using the mobile nylon bag technique. 56

IV.3 Ruminal and intestinal crude protein disappearance (%) of total diet (TD) samples using the mobile nylon bag technique. 57

IV.4 Ruminal and intestinal dry matter disappearance (%) of protein sources (PS) using the mobile nylon bag technique. 58

IV.5 Ruminal and intestinal dry matter disappearance (%) of total diets (TD) using the mobile nylon bag technique. 59

IV.6 Comparison of crude protein (CP) and dry matter (DM) digestibility estimates for total diet (TD) obtained using the mobile nylon bag technique, and the total fecal collection method. 60

V.1 Intake and Digestion of Dry Matter (DM) and crude protein (CP) as Measured by Total Fecal Collection (TFC) or Dysprosium (Dy) and percent recovery of Dy. 68

V.2 Variation in digestibility coefficients as determined using the dysprosium ratio technique on four fecal grab samples collected over a 24 hr period. 69

List of Figures

Figure — Page

II.1 Dry Matter Disappearance (%) From Nylon Bags As A Function Of Time When Samples Of The Protein Source Were Incubated 13

II.2 Protein Disappearance (%) From Nylon Bags As A Function Of Time When Samples Of The Protein Source Were Incubated 14

I. INTRODUCTION

The crude protein (CP) and digestible crude protein (DCP) systems are recognized as being seriously deficient as methods of evaluating the quality of feedstuffs for ruminant livestock. This has led to the development of new protein feeding systems (Jarrige et al. 1978; Black et al. 1980; Van Soest et al. 1982; Agricultural Research Council 1985; National Research Council 1985) which attempt to take into account the influence of the rumen environment on the supply and quality of protein to the small intestine and subsequently to the tissues.

Historically, the CP system had the advantage of being exceedingly simple in that only the nitrogen (N), or CP (CP=Nx6.25), content of the diet or individual feed ingredients was required to determine the CP value (National Research Council 1978). However, rations containing the same level of CP from different feed sources will not always support equal levels of productivity. Further, non-protein-nitrogen (NPN) and true protein were not differentiated by the CP system, nor did the system consider the variation between and within protein sources in meeting rumen microbial protein requirements and subsequent protein supply to the small intestine.

The new protein systems dictate that a sufficient supply of rumen degradable protein (RDP) is available, not only to meet the requirement of the rumen microbes, but to facilitate optimal ruminal fibre digestion. In addition, the diet should contain sufficient rumen undegradable protein (UDP) to meet any additional protein requirements of the animal in excess of that supplied by microbial protein.

Several in vitro methods for determining ruminal protein degradability have been evaluated in recent years (Broderick 1982; Poos-Floyd et al. 1985). A significant proportion of this research has focussed on the in situ or in sacco technique. The in situ technique differs from the typical in vitro digestion procedure in that feed samples are suspended in nylon bags within the rumen, thus simulating a normal rumen environment. Protein degradability is calculated on the basis of rate of protein disappearance from the bag as a function of ruminal

fractional outflow rate (Ørskov and McDonald 1979). It must be recognized, however, that the in situ nylon bag technique does not take into account repeated feed processing by the animal, ie. mastication, mixing with saliva etc., which would normally occur before the feed is exposed to rumen microbes. In addition, the in situ technique simply measures the movement of feed protein from the bag to the outside environment. Rate of disappearance from nylon bags is not necessarily synonymous with rate of protein degradation in vivo. To provide true estimates of protein degradation a measure of the kinetics of particle movement is required. These values are difficult to accurately determine (Gill et al. 1984). Further, estimates of the effective degradability of feed protein by the in situ technique cannot necessarily be compared directly to in vivo estimates. However, they are valuable as relative estimates of the RDP content of various feedstuffs.

A simple laboratory procedure for routine evaluation of ruminal protein degradability which would take into account the kinetics of particulate movement through the rumen has yet to be developed. The use of proteolytic enzymes to measure protein catabolism (Krishnamoorthy et al. 1983) is of considerable interest. However, the results are variable when compared to other in vitro methods (Poos-Floyd et al. 1985).

In vivo estimates of the total non-ammonia N reaching the small intestine are the sum of UDP, rumen microbial protein and endogenous protein. Undegraded dietary protein is determined by calculating the total protein reaching the duodenum minus microbial protein, ammonia and endogenous protein. Undegraded dietary protein includes feed protein which is resistant to ruminal microbial degradation, as well as any potentially degradable feed protein that escaped rumen degradation. Rumen microbial protein production is subject to adequate amounts of all nutrients, including preformed amino acids and peptides being supplied (Cotta and Hespell 1984). Although a variety of microbial markers have been evaluated, the results are disappointing due to lack of agreement among methods for measuring microbial protein production within the same experiment (Tamminga 1978). This suggests that, although it may be reasonable to compare treatments using a specific marker within an experiment, the same

comparison among experiments may produce conflicting results, reflecting methodological differences. Endogenous protein contribution has been recently estimated at 15.8 g N/day in non-lactating dairy cows nourished by intragastric infusion (Hovell et al. 1984). It is generally assumed that endogenous N secretions will vary with intake (National Research Council 1985).

A critical calculation in the determination of protein quality is the intestinal digestibility or disappearance of the undegraded protein fraction. Determination of this value in vivo is difficult because of the problems associated with separation of endogenous, microbial, and feed N in ileal digesta samples. Historically, these values have been estimated using regression analysis (Faichney and White 1979; Zinn and Owens 1982) or digestion of the UDP fraction by rats (Rooke et al. 1981). However, these estimates only provide values for the entire diet as opposed to individual feed ingredients.

Recently, estimates of the intestinal availability of UDP for total diets and specific protein sources have been obtained using a technique known as the modified or mobile nylon bag technique (Kirkpatrick and Kennelly 1984; Hvelplund 1985; de Boer et al. 1986; de Boer et al 1987). Briefly the technique involves incubating samples of feed in the rumen for various lengths of time in 3.5 x 5.5 cm nylon bags. The bags are then incubated in a pepsin-HCL bath or inserted directly into the duodenum and recovered in the feces. Results using this technique suggest that the intestinal digestibility of the UDP fraction of feedstuffs can be determined simply and reliably.

An ongoing problem in dairy cattle research has been the lack of a suitable indicator of diet digestibility for use with lactating dairy cattle in confinement and loose housing conditions. Conventional internal indicators such as lignin and acid insoluble ash are variable and incomplete in percent recovery (Block et al. 1981; Muntifering 1982), while standard total fecal collection techniques are time consuming and labour intensive, with the additional complication of urine diversion.

Rare earth elements have a high affinity for feed particles (Combs et al. 1984; Beever and Ellis 1985) and appear to be unabsorbed in the ruminant digestion process (Ellis 1968; Young et al. 1976). Dysprosium, a rare earth element, has been suggested as a convenient indicator of diet digestibility in cattle (Ellis 1968).

The objectives of the studies described herein were to evaluate the quality of several locally available protein sources in the diets of pregnant heifers. The protein sources and their respective total diets were examined in terms of their ruminal degradability, intestinal protein supply and availability of the protein supplied to the small intestine. In addition, the efficacy of dysprosium as a digestibility marker for ruminants was examined.

A. BIBLIOGRAPHY

AGRICULTURAL RESEARCH COUNCIL. 1985. The Nutrient Requirements of Ruminant Livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Farnham Royal, England.

BEEVER, D.E. and ELLIS, W.C. 1985. Binding of rare earths to specific feed particles. Proc. Nutr. Soc. 44: 117A.

BLACK, J.L., BEEVER, D.E., FAICHNEY, G.H., HOWARTH, B.R. and GRAHAM, N. 1980. Simulation of the effects of rumen function on the flow of nutrients from the stomach of sheep: Part 1 - Description of a computer program. Agric. Systems 6: 195-204.

BLOCK, E., KILMER, L.H. and MULLER, L.D. 1981. Acid insoluble ash as a marker of digestibility for sheep fed corn plants or hay and for lactating dairy cattle fed hay ad libitum. J. Anim. Sci. 52: 1164-1170.

BRODERICK, G.A. 1982. Estimation of protein degradation using in situ and in vitro methods. Pages 72-80 *in* F.N. Owens, ed. Protein requirements for cattle: Symposium. MP-109, Div. of Agriculture, Oklahoma State University, Stillwater, Okla.

COMBS, D.K., SHAVER, R.D., SINGH, N. and SATTER, L.D. 1984. Effects of application procedure, pH and microbial growth on retention of ytterbium and cerium by brome hay. Can. J. Anim. Sci. (Suppl.) 64:66-67.

COTTA, M.A. and HESPELL, R.B. 1984. Protein and amino acid metabolism of rumen bacteria. Pages 122-138 in L.P. Milligan, W.L. Grovum, and A. Dobson, eds. Control of digestion and metabolism in ruminanats. Prentice-Hall, Englewood Cliffs, New Jersey.

DE BOER, G., MURPHY, J.J., KENNELLY, J.J. and KIRKPATRICK, B.K. 1986. The mobile nylon bag for estimation of intestinal availability of rumen undegradable protein. J. Dairy Sci. (Suppl) 69: 1196.

DE BOER, G., MURPHY, J.J. and KENNELLY, J.J. 1987. Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977-982.

ELLIS, W.C. 1968. Dysprosium as an indigestible marker and its determination by radioactivation analysis. J. Agr. Food Chem. 16: 220-224.

FAICHNEY, G.J. and WHITE, G.A. 1979. Formaldehyde treatment of concentrate diets for sheep. 3. Absorption of amino acids from the small intestine. Aust. J. Agric. Res. 30: 1163-1175.

GILL, M., FRANCE, J., SIDDONS, R.C. and DHANOA, M.S. 1984. Compartmental models incorporating particle size for the estimation of digestion in the rumen. Techniques in Particle Size Analysis of Feed and Digesta in Ruminants. Pages 142-153 *in* P.M. Kennedy, ed. Can. Soc. of Anim. Sci. Occasional Publication No.1.

HOVELL, F.D. DE B., ASPLUND, J.M., ØRSKOV, E.R., VIERA, J.K. and MACLEOD, N.A. 1984. Basal and fasting nitrogen losses and endogenous flow of nitrogen of ruminants nourished by intragastric infusion. Can. J. Anim. Sci. (Suppl.) 64: 142-143.

HVELPLUND, T. 1985. Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep by an in sacco procedure. Acta Agric. Scand. (Suppl.) 25. In Press.

JARRIGE, R., JOURNET, M. and VERITE, R. 1978. Azote. n Alimentationdes Ruminants. Pages 89-94 *in* R. Jarrige, eds. INRA Versailles, France.

KIRKPATRICK, B.K. and KENNELLY, J.J. 1984. Prediction of digestibility in cattle using a modified nylon bag technique. Can. J. Anim. Sci. 64: 1104.

KRISHNAMOORTHY, U.X., SNIFFEN, C.J., STERN, M.D. and VAN SOEST, P.J. 1983. Evaluation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. J. Dairy Sci. 65: 217-225.

MUNTIFERING, R.B. 1982. Evaluation of various lignin assays for determining ruminal digestion of roughages by lambs. J. Anim. Sci. 55: 432-437.

NATIONAL RESEARCH COUNCIL. 1978. Ruminant nitrogen usage. National Academy Press, Washington, D.C., U.S.A.

NATIONAL RESEARCH COUNCIL. 1985. Ruminant nitrogen usage. National Academy Press, Washington, D.C., U.S.A.

ØRSKOV, E.R. and MCDONALD, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. Camb. 92: 499-503.

POOS-FLOYD, F.M., KLOPFENSTEIN, T. and BRITTON, R.A. 1985. Evaluation of laboratory techniques for predicting ruminal protein degradation. J. Dairy Sci. 68: 829-839.

ROOKE, J.A., SEYMOUR, S.J. and ARMSTRONG, D.G. 1981. The effect upon the nutritional value of soybean meal of incubation in the bovine rumen. Proc. Nutr. Soc. 40: 82A.

TAMMINGA, S. 1978. Measurement of microbial protein synthesis in the rumen. Pages 5.1-5.11 *in* D.F. Osbourn, D.E. Beever and D.J. Thomson, eds. Rumen fermentation and feed evaluation.

VAN SOEST, P.J., SNIFFEN, C.J., MERTENS, D.R., FOX, D.G., ROBINSON, P.H. and KRISHNAMOORTHY, U.X. 1982. A net protein system for cattle: The rumen submodel for nitrogen. Pages 265-279 *in* F.N. Owens, ed. Protein requirements for cattle: Symposium. MP-109, Div. of Agriculture, Oklahoma State University, Stillwater, Okla.

YOUNG, M.C., THEURER, B., OGDEN, P.R., NELSON, G.W. and HALE, W.H. 1976. Dysprosium as an indicator in cattle digestion trials. J. Anim. Sci. 58: 1270-1279.

ZINN, R.A. and OWENS, F.N. 1982. Predicting net uptake of non ammonia nitrogen from the small intestine. Pages 133-140, *in* F.N. Owens, ed. Protein requirements for cattle: Symposium. MP-109, Div. of Agriculture, Oklahoma State University, Stillwater, Okla.

II. IN SITU DEGRADABILITY OF PROTEIN AND DRY MATTER FROM SINGLE PROTEIN SOURCES AND FROM A TOTAL DIET [1].

A. INTRODUCTION

Extent of feed protein degradation in the rumen has been the focal point of proposed protein feeding systems for ruminants (Jarrige et al. 1978; Black et al. 1980; Van Soest et al. 1982; ARC 1985). The systems are based on the recognition that ruminal microbial protein synthesis is not adequate to meet the N requirements of rapidly growing ruminants or lactating dairy cows. The duodenal amino acid needs of high producing ruminants are met by a combination of microbial protein and ruminal undegradable feed protein (UDP). Dietary protein requirements for ruminants therefore, are best expressed in terms of UDP and ruminal degraded protein (RDP). The most efficient use of dietary N occurs when the correct balance of UDP and RDP is fed (Murphy and Kennelly 1986; Murphy et al. 1986). Currently, a major factor limiting the widespread application of feed formulation based on RDP and UDP is the absence of reliable data on these parameters for many feedstuffs under differing levels of protein and dry matter intake.

The in situ nylon bag technique has been suggested as an alternative to the in vivo technique (Mehrez and Ørskov, 1979) for estimating crude protein (CP) disappearance from the rumen. Degradability estimates using the in situ nylon bag technique have proven reliable in ranking individual protein sources relative to in vivo estimates (Stern and Satter 1984).

Although meat and bone meal (MBM) is not a traditional ingredient in the diets of ruminant animals, it is of considerable interest because of its low degradability (10-30%) in vitro (ARC 1985), and in vivo (Zinn et al. 1981). This may be the result of current processing techniques (vat drying), which involves heating via steel pipes, where prolonged exposure to high temperatures can occur.

---

[1]This chapter has been previously published. Kirkpatrick B. K. and J. J. Kennelly. J. Anim. Sci. 1987. 65:567-576.

The objectives of studies reported herein were to examine the in situ DM and CP degradation of both protein sources and the total diets in which they were incorporated. Samples were incubated in the rumen of cattle fed diets which differed in both source and level of protein. Dry matter and CP disappearance was estimated by fitting the data to nonlinear equations.

B. MATERIALS AND METHODS

Six pregnant Holstein heifers (300 to 350 kg bodyweight) fitted with permanent ruminal and duodenal cannulae were assigned in a 6x6 Latin square to six dietary treatments. Experimental diets were based on chopped brome alfalfa hay and barley (control) supplemented with canola meal (CM) soybean meal (SBM) or meat and bone meal (MBM). Diets (Table II.1) were designed to compare these four protein sources in addition to CM and SBM at two dietary CP levels. The six concentrate mixtures (dry basis) were: barley 14% CP (B14), barley/canola meal 16.5% CP (CM16.5), barley/soybean meal 16.5% CP (SBM16.5), barley/meat and bone meal 16.5% CP (MBM16.5), barley/canola meal 19% CP (CM19) and barley/soybean meal 19% CP (SBM19). Diets CM16.5, SBM16.5 and MBM16.5 were equivalent on a CP basis, whereas on a calculated UDP equivalent basis (ARC 1984) MBM16.5, CM19 and SBM19 were similar. Heifers were fed 8.5 kg of a 50:50 hay/concentrate (dry basis) mix in 12 equal amounts each day at 2-h intervals.

All feeds (obtained commercially) were ground through a 2-mm screen before being placed in nylon bags. The MBM product was commercially processed by vat drying (boiling in oil) at 250 C rather than the more common process of heating by steel pipes. Samples of protein source and total diet were only incubated in the rumen of heifers only wherein they constituted a component of the diet. For example, CM was incubated in both CM16.5 and CM19.

Bags were made of nylon cloth[2] with an average mesh size of 48μm. The nylon cloth

[2]B and SH Thompson and Co. Ltd., Montreal.

(20 cm x 15 cm) was folded in half and sewn with a double row of stitches with rounded corners to allow easy removal of particulate material. The final bag size exposed to ruminal fermentation was approximately 7x10 cm. Approximately 5 g (air dry) of test proteins were placed in nylon bags which were tied shut with a nylon string. A total of 14 bags per treatment were tied at the end of a 70-cm main line, which was weighted with a sand filled bottle and suspended in the rumen. The line was secured at the ruminal cannula and the bags were incubated in the ventral sac of the rumen for 1, 3, 6, 9, 12, 15 and 24-h. At the end of each incubation time, two bags were removed randomly from the rumen and washed under running tap water until the rinsing water was colorless (approximately 3 min). An additional two bags per treatment, washed by the procedure outlined above, were used for zero-hour (0-h) values. Washed nylon bags were dried in a forced-air drying oven at 65 C for 48-h. The contents of each bag were subjected to Kjeldahl N analysis (AOAC 1980). The percent disappearance of DM and CP at each incubation time was calculated from the proportion remaining after incubation in the rumen. The disappearance rate was fitted to the following equation (Ørskov and McDonald 1979):

$$P = a + b(1 - e^{-kt}),$$

where P = disappearance rate at time t, a = an intercept representing the portion of DM or CP solubilized, b = the fraction of DM or CP which will be degraded when given sufficient time for digestion in the rumen, k = a rate constant of disappearance of fraction b, and t = time of incubation. Nonlinear parameters a, b and k were estimated by an iterative least-square procedure and best fit values were chosen using the smallest sums of squares after 10 iterations. While the above equation gives CP degradability at given incubation times, it does not predict the amount of protein which will actually be degraded in the rumen (effective degradability). Thus, further attempts have been made in this study to calculate effective degradability of DM (EDDM) and CP (EDCP) by the following equation (Ørskov and McDonald 1979)

$$\text{EDDM or EDCP} = a + ((bxk)/(k+r))$$

where r is the estimated rate of outflow from the rumen.

Data were subjected to analysis of variance. Period effects were not significant ($P>0.05$); therefore, treatments were tested for significance against animal x test protein. When F values were significant ($P<0.05$), treatment means were compared at probability level of 0.05 using Student-Newman-Keuls test (Steel and Torrie 1960). Estimated effective degradability of DM and effective degradability of CP for CM16.5, CM19 and SBM16.5 and SBM19 were subject to analysis via orthogonal contrasts in order to evaluate within protein differences (Steele and Torrie 1960).

C. RESULTS AND DISCUSSION

Dry Matter and Crude Protein Disappearance from Protein Sources. Dry matter and CP disappearance values of protein source as a function of ruminal incubation time are summarized in table 2. Dry matter disappearance of MBM in heifers fed MBM16.5 was lower ($P<0.05$) than DM disappearance from all other protein sources. Soybean meal samples in SBM16.5 tended to have a higher DM disappearance than CM samples in CM16.5, with significance ($P<0.05$) at 0, 1 and 24-h. Results were similar for SBM samples in SBM19 which had higher ($P<0.05$) degradability at 1, 12 and 24-h than CM samples in CM19. Dry matter disappearance of barley samples in B14 was consistently higher than all other diets. Within protein source at differing CP levels (CM16.5 vs CM19 and SBM16.5 vs SBM19) a consistent trend of increasing DM disappearance, at higher protein levels, was noted; SBM19 being greater than SBM16.5 ($P<0.05$) at 24-h.

Disappearance of CP in SBM16.5 and CP in MBM16.5 was less ($P<0.05$) than observed for other test samples. Soybean meal in SBM16.5 tended to be less degradable than MBM in MBM16.5 at early incubation times. Within protein source at different CP levels (CM16.5 vs CM19.5 and SBM16.5 vs SBM19) there was a consistent trend for increased CP disappearance at higher protein levels. While not significant, CP disappearance of CM in CM19 tended to be higher than in CM16.5. Crude protein disappearance of SBM in SBM19

was greater ($P<0.05$) than SBM in SBM16.5, at 12 and 24-h and tended to be higher at all other times considered. In contrast to results observed for DM, CP disappearance from CM was consistently higher than SBM. Crude protein disappearance of barley in B14 was not different from either of the CM-based diets.

Evaluation of the DM and CP disappearance data for the protein source shows that the initial 0-h wash did not appear to generate major differences, but after 1-h of incubation each of the test feeds differed ($P<0.05$). Weakley et al. (1983) suggest that the major differences in disappearance rates from nylon bags due to particle size would be established in the first hour. Our data support this conclusion.

Within protein sources, DM and CP losses were similar to other data (Ha and Kennelly 1984; Vik-Mo and Lindberg, 1985). Dry matter and CP disappearance curves for all protein sources were similar though distinct in shape (figures II.1 and II.2). Visual appraisal of the DM disappearance curves suggests that for MBM, DM disappearance is relatively constant; barley is highly curvilinear; and CM and SBM (at both protein levels) are intermediate. Crude protein disappearance curves were less distinct, particularly at early incubation times. Meat and bone meal was lowest at later incubation times, CM and SBM diets (at both protein levels) were intermediate, and barley was highest. These estimates are in agreement with previously reported disappearance curves for barley and SBM (Vik-Mo and Lindberg 1985), MBM (Loerch et al. 1983) and CM (Ha and Kennelly 1984).

Dry Matter and CP Disappearance of Total Diets. Dry matter and CP disappearance values of total diet as a function of ruminal incubation time are summarized in table II.3. With the exception of lower ($P<0.05$) DM disappearance of MBM16.5 at 12-h, DM disappearance of total diets were not affected by source or level of dietary CP.

Differences in CP disappearance for total diets followed those seen when protein sources were incubated (Table II.3). Diets did not show any differences in CP disappearance at 1, 9 and 15-h. Interestingly, SBM16.5 was lowest in degradability at all times except 0 and

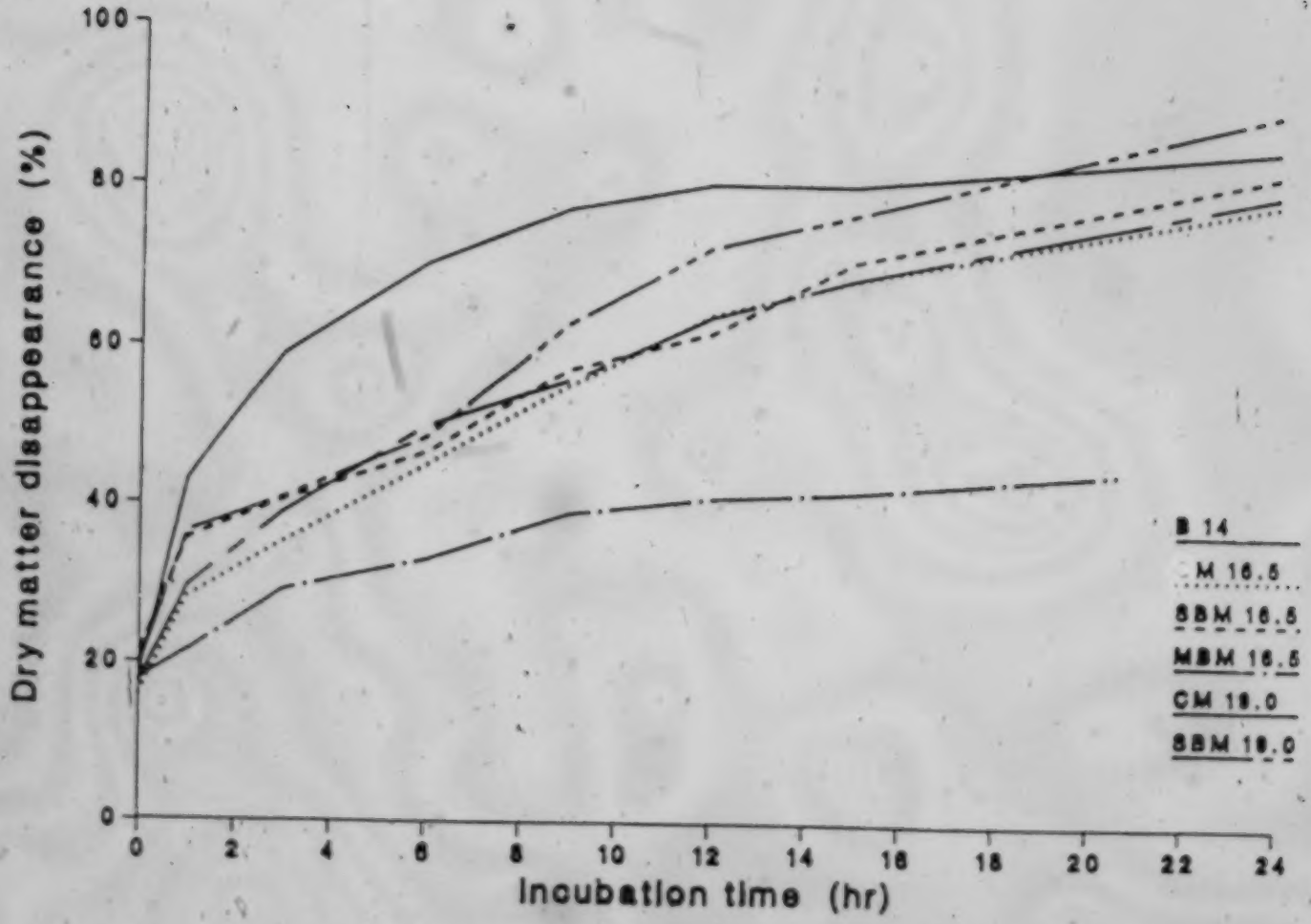

Figure II.1 Dry Matter Disappearance (%) From Nylon Bags As A Function Of Time When Samples Of The Protein Source Were Incubated

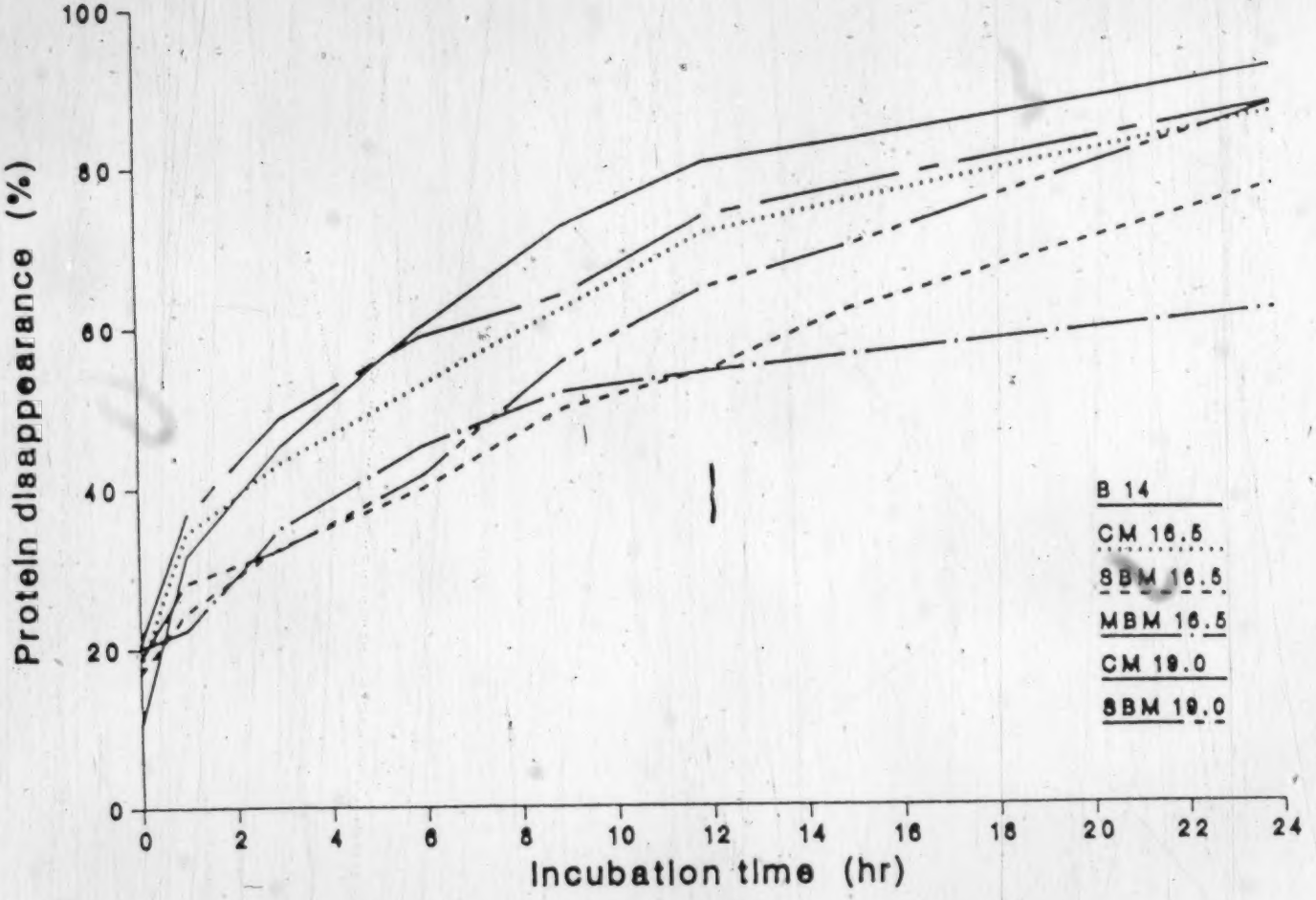

Figure II.2 Protein Disappearance (%) From Nylon Bags As A Function Of Time When Samples Of The Protein Source Were Incubated

24-h. Within protein sources at different dietary levels (CM16.5 vs CM19 and SBM16.5 vs SBM19) samples in the higher protein diets tended to be more degradable.

Dry matter and CP disappearance curves were relatively indistinguishable when samples of the various total diets were studied. Differences in initial (0-h) wash were observed only between MBM16.5 and SBM19.5.

Effective Degradability of DM and CP for Protein Sources. Effective degradabilities of DM and CP for the protein sources studied are summarized in table II.4. Meat and bone meal in MBM16.5 had a lower ($P<0.05$) soluble DM fraction (parameter a) than all other protein sources. The soluble N fraction (parameter a) for MBM in MBM16.5 was equivalent to that observed for SBM in SBM16.5 and SBM19, higher than barley in B14, and lower than CM in CM16.5 and CM19. The potentially degradable fraction for both DM and CP (sum of parameters a + b) was least ($P<0.05$) for MBM in MBM16.5 and greatest for SBM in SBM16.5 and SBM19. The fractional rate constant (k) of both DM and CP was intermediate for MBM in MBM16.5 and least for CM- and SBM-based diets. Effective degradability of dry matter was lower ($P<0.05$) for MBM in MBM16.5 than for the other protein sources. The rapidly degradable N fraction (a) was higher for CM than SBM at both 16.5 and 19% CP levels (18.6 vs 15.5 and 22.2 vs 14.2, respectively). The sum of the fractions a and b were greater than 100%. It has been suggested that large b values may be due to disproportionate disappearance of CP at 24-h (Ha and Kennelly 1984). Effective degradability of CP for CM and SBM tended to increase when percent protein in the diet increased (63.2, CM16.5 vs 72.0, CM19 and 60.2, SBM16.5 vs 64.4, SBM19). Analyses of the CM and SBM results, via orthogonal contrasts (Steele and Torrie 1960), show an increase ($P<0.05$) in effective degradability of CP for CM19 when compared to CM16.5. Recent work suggests that SBM may exhibit an adaptive interaction to the occurrence of SBM (Vik-Mo and Lindberg 1985) and other protein sources (Loerch et al. 1983) in the basal diet. Although results obtained in this experiment were not always significant, there was a definite trend for increased effective degradability of CP of the

protein source as CP level in the diet increased. Barrio et al. (1985) has suggested that this effect may be further confounded by the ratio of concentrate to forage in the basal diet, however, this should have little consequence in practical feeding situations for high producing dairy cows.

Effective CP degradability values indicate that MBM in MBM16.5 was most resistant to microbial attack. Low ruminal degradability values for MBM have been reported extensively in the literature (Stock et al. 1981; Ørskov 1982; Barrio et al. 1985). Stock et al. (1981) correlated low and variable degradabilities for MBM with high acid detergent insoluble N (ADIN) values, suggesting the protein may be damaged or insoluble (e.g. keratin). In this experiment, ADIN values for MBM were so low as to be virtually nondetectable. Effective degradability of CP of MBM appears not to be influenced by the occurrence of MBM in the basal diet (Loerch et al. 1983) or by forage:concentrate ratio (Barrio et al. 1985).

Effective Degradability of DM and CP of Total Diets. The nonlinear parameters for effective degradability of DM and effective degradability of CP for total diet are summarized in table II.5. Effective degradability of DM and effective degradability of CP of the diet were influenced by the source of supplemental protein, particularly for MBM and SBM diets. Effective degradability of DM and effective degradability of CP values for MBM16.5 were lower ($P<0.05$) than all other diets. The effective degradability of DM rate constant (k) for B14 was ($P<0.05$) higher than other diets. There were no differences among diets for a and b. Effective degradability of DM and effective degradability of CP values obtained in this study correlate well with published values (Stern and Satter, 1984).

Fractional Outflow Rates. Effective degradability of DM and effective degradability of CP estimates were calculated assuming that protein sources would not influence solid outflow rates from the rumen. Variation in particle size among protein supplements in the diet may affect fractional outflow rates. However, Eliman and Ørskov (1985) found there were no difference between coarse (9.5 mm) and fine (1.5 mm) particles

for SBM or fish meal. Although many factors such as physiological status (Gonzalez et al. 1985), environmental temperature (Kennedy et al. 1976) and frequency of feeding (Ørskov 1982) can influence outflow rates from the rumen, different protein sources at the same level of feeding are not expected to change outflow rates to any extent (Ørskov 1982). It has been suggested that within a given experiment, ranking of protein sources should not change at various fractional outflow rates (ARC 1984). Effective degradability of CP values for the protein source studied at five fractional outflow rates are in table II.6. Protein degradability values, particularly SBM (16.5 and 19), were affected by outflow rates. Effective degradability of CP ranks CM in CM16.5 lower than SBM in SBM16.5 at .04 h^{-1} outflow while at .07 h^{-1} the ranking was reversed. Between .04 h^{-1} and .06 h^{-1} effective degradability of CP of both SBM diets changed by approximately 10 units while CM16.5 changes by approximately 7 units. Effective degradability of CP values at fractional outflow rates used in this experiment are in agreement with ARC (1985).

D. GENERAL DISCUSSION

Meat and bone meal, although not a traditional ingredient in dairy cattle diets, was considered in this study because it is a good source of DP and furthermore the method of processing used resulted in low acid detergent insoluble N (ADIN) levels. There were no palatability problems in the feeding of this product. Research by Craig and Broderick (1984) suggests that MBM may actually have an improved essential amino acid profile after microbial attack in the rumen. In vitro studies indicate that a disproportionate release of nonessential amino acids during ruminal degradation results in an enhanced profile of essential amino acids moving to the small intestine. If properly processed, then, MBM may be an effective source of UDP for dairy cattle.

Ruminal protein degradability is a function of the proteolytic activity of the diet in question. It has been suggested (McAllan and Smith 1983) that as cellulolytic bacteria are partially dependent on a supply of preformed amino acids and peptides, degradable fractions

of protein sources that provide appropriate substrates would evoke a stronger bacterial response. The result as suggested by Barrio et al. (1985) is that as dietary parameters are changed the differences are not due to chemical characteristics of the substrate in question but rather to shifts in microbial activities. The result may be an adaptive dietary influence on ruminal protein degradability (Loerch et al. 1983). Vik-Mo and Lindberg (1985) suggest that increases in protein level in the basal diet (15 vs 25%) induce an increase in ruminal protein degradability. In the study reported herein, protein degradability tended to increase with increasing protein level in the diet. However, this effect was not consistent across protein sources or ruminal incubation time.

It is very difficult to obtain absolute degradability values for protein source sources or total diet (Stern and Satter 1984). It is more realistic therefore, to determine relative RDP and UDP values. These values rank protein sources and diets relative to one another under specific feeding conditions. In this experiment, assuming a fractional outflow rate of .05 h^{-1} MBM CP was least degradable, SBM was intermediate and CM had highest effective degradability of CP value. These results are valid both for the individual protein sources and the total diets.

In situ CP degradability values will be affected by the characteristics of the feed in question, experimental technique (Setala 1983) and the occurrence of other dietary components (Loerch et al. 1983). In order to accurately evaluate the UDP and RDP fractions of available protein sources, the degradability values should be determined under similar physiological and feeding conditions to which they are to be applied.

Table II.1 Formulation and Composition of Experimental Diets (%) Dry Matter Basis.

Item	Diets[a]					
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19
Ingredients						
Alfalfa-brome hay	50.0	50.0	50.0	50.0	50.0	50.0
Barley (B)	46.3	34.3	36.8	36.8	25.0	31.0
Canola meal (CM)		12.0			21.3	
Soybean meal (SBM)			9.5			15.3
Meat and bone meal (MBM)				9.5		
Molasses	1.5	1.5	1.5	1.5	1.5	1.5
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0
TM salt[b]	0.25	0.25	0.25	0.25	0.25	0.25
Chemical analyses						
Crude protein	13.7	16.2	16.5	16.6	19.0	19.4
Acid detergent fiber	26.4	31.0	24.5	24.9	27.2	26.4
Calcium	0.86	0.93	0.96	1.77	1.14	1.01
Phosphorus	0.56	0.63	0.68	0.92	0.87	0.83
Ash	6.1	6.4	6.1	7.9	7.6	7.0

[a] All diets were fortified on a per kg basis with Vitamin A palmitate 4000 IU; D 460 IU; D, 123.7 IU; Vitamin E 67 IU.

[b] Trace mineral salt contains - Se 25 ppm, Co 40 ppm, Mg 0.35%, Cu 0.25%, I 0.01%, Zn 0.75%.

Table II.2 Dry Matter and Crude Protein Disappearance (%) From Nylon Bags as a Function of Time, When Samples of the Protein Source Were Incubated in the Rumen of Heifers Fed Diets Containing the Test Protein Source.

Incubation time, hr	Protein source incubated[a]						
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[b]
Dry matter							
0	18.7^{cd}	15.8^{c}	21.5^{d}	17.9^{cd}	17.5^{cd}	19.4^{cd}	1.1
1	43.1^{e}	28.3^{d}	35.7^{c}	21.6^{f}	29.7^{d}	36.5^{c}	1.5
3	59.1^{c}	35.4^{d}	40.5^{d}	29.4^{e}	39.0^{d}	40.8^{d}	1.8
6	70.3^{c}	45.0^{d}	46.5^{d}	33.3^{e}	49.8^{d}	48.4^{d}	2.4
9	77.5^{c}	55.3^{d}	57.5^{d}	39.2^{e}	56.0^{d}	63.2^{d}	2.4
12	80.8^{c}	64.6^{d}	62.3^{d}	41.3^{e}	64.3^{d}	72.9^{d}	2.8
15	80.8^{c}	69.0^{c}	71.3^{c}	42.2^{d}	69.1^{c}	77.2^{c}	2.8
24	86.0^{c}	79.3^{d}	82.9^{d}	46.8^{e}	80.4^{d}	90.8^{c}	2.3
Crude Protein							
0	10.4^{c}	17.6^{d}	16.9^{d}	20.1^{d}	20.6^{d}	18.8^{d}	2.7
1	31.8^{cd}	34.5^{c}	24.6^{ef}	22.4^{f}	36.7^{c}	28.3^{d,e}	1.5
3	45.4^{c}	43.3^{c}	32.9^{d}	35.2^{d}	48.9^{c}	32.6^{d}	1.7
6	60.3^{c}	52.9^{cd}	40.1^{e}	45.3^{d,e}	59.1^{c}	41.7^{d,e}	3.2
9	73.0^{c}	62.6^{c,d}	49.9^{e}	52.0^{e}	64.4^{c,d}	55.9^{d,e}	2.9
12	81.1^{c}	72.3^{c,d}	54.6^{e}	54.5^{e}	74.3^{c,d}	65.4^{d}	3.0
15	84.0^{c}	76.2^{c,d}	62.6^{e,f}	56.6^{f}	78.1^{c,d}	70.6^{d,e}	3.1
24	93.5^{c}	87.7^{c}	78.4^{d}	62.8^{e}	89.0^{c}	88.7^{c}	2.6

[a] Barley (B) was incubated in animals fed B14; canola meal (CM) in CM16.5 and CM19; soybean meal (SBM) in SBM16.5 and SBM19 and meat and bone meal (MBM) in MBM16.5.

[b] Standard error of the mean.

^{c,d,e,f} Means in the same row with different letter in their superscripts differ ($P<0.05$).

Table II.3 Dry Matter and Crude Protein Disappearance (%) From Nylon Bags as a Function of Time When Samples of the Total Diet Were Incubated In the Rumen of Heifers Fed Diets Containing the Test Protein Source.

Incubation time, hr	Total diet incubated[a]						
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[b]
Dry matter							
0	31.0^{c}	34.5^{d}	33.9^{d,e}	31.7^{c,d,e}	32.1^{c,d,e}	32.2^{c,e}	0.6
1	40.5	37.6	38.6	36.4	38.6	40.2	1.8
3	51.0	42.7	44.9	42.3	46.0	44.5	2.2
6	54.5	51.3	49.8	49.6	52.0	51.4	2.2
9	60.6	54.8	56.5	54.0	57.2	57.4	1.8
12	65.0^{c}	58.9^{c,d}	59.5^{c,d}	53.2^{d}	60.4^{c,d}	62.9^{c,d}	2.5
15	64.2	63.2	61.5	59.9	63.7	64.4	1.8
24	69.1	65.3	69.9	64.6	69.2	71.7	2.1
Crude protein							
0	35.1^{c}	34.8^{c}	29.7^{d}	29.9^{d}	28.2^{d}	28.5^{d}	1.0
1	43.7	46.6	38.7	41.6	45.1	43.4	2.0
3	56.1^{c}	52.9^{cd}	44.1^{d}	48.6^{cd}	55.0^{c}	50.6^{cd}	2.6
6	58.1^{cd}	60.7^{cd}	48.5^{d}	56.8^{cd}	64.5^{c}	58.1^{cd}	2.9
9	60.2	64.6	66.0	70.1	70.4	72.0	2.7
12	67.5^{c}	66.0^{c}	71.2^{cd}	74.6^{cd}	76.1^{cd}	79.2^{d}	3.0
15	76.4	77.2	69.1	69.4	78.7	79.6	2.8
24	81.1^{cd}	81.4^{cd}	81.4^{cd}	77.0^{d}	85.4^{c}	87.5^{c}	1.9

[a] Samples of each total diet were incubated in animals being fed that diet; B, barley; CM, canola meal; SBM, soybean meal; MBM, meat and bone meal.

[b] Standard error of the mean.

[c,d,e] Means in the same row with different letters in their superscripts differ ($P<0.05$).

Table II.4 Nonlinear Parameters and Effective Degradability of Dry Matter (EDDM) and Crude Protein (EDCP) When Samples of the Protein Source Were Incubated in the Rumen of Animals Fed Diets Containing in Test Protein.

	Diet and protein source incubated[a]						
Parameter[b]	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[c]
Dry matter							
a	21.3[d]	18.2[d]	25.6[e]	17.6[d,f]	21.1[d]	23.9[d,e]	1.04
b	61.1[d]	71.9[d]	76.3[d]	29.7[e]	74.5[d]	76.1[d]	17.18
k	0.3[d]	0.09[e]	0.07[e]	0.1[d,e]	0.09[e]	0.09[e]	.024
EDDM	74.1[d]	63.0[e]	66.6[d,e]	39.0[f]	64.2[e]	69.4[d,e]	2.05
Crude Protein							
a	11.6	18.6	15.5	14.6	22.2	14.2	6.81
b	82.0[d,e]	77.1[d,e]	103.0[e]	47.5[d]	83.1[d,e]	99.4[e]	11.64
k	0.2	0.09	0.06	0.1	0.1	0.1	.022
EDCP	73.1[b]	63.2[b]	60.2[b]	45.9[c]	72.0[b]	64.4[b]	3.45

[a]Barley was incubated in animals fed B14; canola meal in CM16.5 and CM19; soybean meal (SBM) in SBM16.5 and SBM19 and meat and bone meal (MBM) in MBM16.5.

[b]a, b and k are non-linear parameters. Effective dry matter and crude protein degradability are calculated on the basis of .05 h^{-1} solid outflow rates.

[c]Standard error of the mean..

[d,e,f]Means in the same row with different letters in their superscripts differ ($P<0.05$).

Table II.5 Nonlinear Parameters and Effective Degradability of Dry Matter (EDDM) and Crude Protein (EDCP) When Samples of the Total Diet (TD) Were Incubated.

	Total Diet[a]						
Parameters[b]	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[c]
Dry matter							
a	32.7	34.1	34.9	34.2	34.1	33.9	1.04
b	36.6	39.1	42.3	34.2	37.3	41.7	2.74
k	0.2[d]	0.1[e]	0.1[e]	0.1[e]	0.1[e]	0.1[e]	.019
EDDM	60.7[d]	59.2[d]	59.9[d]	55.8[e]	59.3[d]	61.4[d]	1.10
Crude Protein							
a	36.4[d]	37.7[d]	32.1[e]	32.7[e]	31.5[e]	31.8[e]	1.20
b	48.6[d]	50.3[d]	70.5[e]	44.2[d]	52.3[d]	59.6[e]	4.29
k	0.1[d]	0.1[d]	0.1[e]	0.1[e]	0.2[e]	0.1[e]	.029
EDCP	70.9[d]	71.4[d]	67.7[e]	65.0[e]	72.0[d]	72.8[d]	1.43

[a]Samples of each total diet were incubated in animals being fed that diet; B, barley; CM, canola meal; SBM, soybean meal; MBM, meat and bone meal.

[b]a b and k are non-linear parameters. Effective dry matter and crude protein degradability are calculated on the basis of .05 h^{-1} solid outflow rates.

[c]Standard error of the mean.

[d,e]Means in the same row with different letters in their superscripts differ ($P<0.05$).

Table II.6 Effective Degradability of Crude Protein at Different Fractional Outflow Rates When Samples of Crude Protein Sources or Total Diet were Incubated.

Item	Degradability at fractional outflow rate, h^{-1}				
	0.02	0.04	0.05	0.06	0.08
Protein source[a]					
B14	83.9	76.3	75.9	70.2	65.1
CM16.5	76.7	66.8	63.4	60.2	55.3
SBM16.5	81.9	65.5	61.4	55.8	49.4
MBM16.5	53.5	48.0	47.4	44.1	41.0
CM19	85.5	75.2	71.8	68.4	63.4
SBM19	84.9	69.7	67.1	60.1	53.4
Total diet[b]					
B14	78.0	73.0	70.9	69.1	66.0
CM16.5	79.5	73.7	71.4	69.4	66.1
SBM16.5	82.2	71.4	67.7	64.7	60.0
MBM16.5	71.2	67.0	65.0	63.3	60.5
CM19	78.3	74.1	72.0	70.3	67.2
SBM19	82.1	75.5	72.8	70.4	66.5

[a]Barley was incubated in animals fed B14; canola meal in CM16.5 and CM19; soybean meal (SBM) in SBM16.5 and SBM19 and meat and bone meal (MBM) in MBM16.5.

[b]Samples of each total diet were incubated in animals being fed that diet.

E. BIBLIOGRAPHY

AOAC. 1980. Official Methods of Analysis. (13th Ed.) Association of Official Analytical Chemists. Washington, D.C.

ARC. 1984. The Nutrient Requrements of Ruminant Livestock. Supplement No 1. Commonwealth Agricultural Bureaux, Farnham Royal, England.

BARRIO, J.R., F.N. OWENS and A.L. GOETSCH. 1985. Soluble nutrients in protein supplements and in situ disappearance. Can. J. Anim. Sci. 65:667.

BLACK, J.L., D.E. BEEVER, G.H. FAICHNEY, B.R. HOWARTH and N. GRAHAM. 1980. Simulation of the effects of rumen function on the flow of nutrients from the stomach of sheep: part 1 - Description of a computer program. Agr. Systems 6:195.

CRAIG, W.M. and G.A. BRODERICK. 1984. Amino acids released during protein degradation by rumen microbes. J. Anim. Sci. 58:436.

ELIMAM, M.E. and E.R. ØRSKOV. 1985. Factors affecting the fractional outflow of protein supplements from the rumen. Anim. Prod. 40:309.

GONZALEZ, J.S., J.J. ROBINSON and C. FRASER. 1985. The effect of physiological state on digestion in the ewe and its influence on the quantity of protein reaching the abomasum. Livstock Prod. Sci. 12:59.

HA, J.K. and J.J. KENNELLY. 1984. In situ dry matter and protein degradation of various protein sources in dairy cattle. Can. J. Anim. Sci. 64:443.

JARRIGE, R., M. JOURNET and R. VERITE. 1978. Azote. In: INRA Publ. (Ed.) Alimentation des Ruminants. pp. 89-128. Versailles-France.

KENNEDY, P.M., R.J. CHRISTOPHERSON and L.P. MILLIGAN. 1976. The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis. Brit. J. Nutr. 36:231.

LOERCH, S.C., L.L. BERGER, D. GIANOLA and G.C. FAHEY, Jr. 1983. Effects of dietary protein source and energy level on in situ nitrogen disappearance of various protein sources. J. Anim. Sci. 56:206.

McALLAN, A.B. and R.H. SMITH. 1983. Effect of dietary nitrogen supplementation on fibre digestion in the rumen. INRA Publ. (Ed.). IV. Int. Symp. Protein Metab. Nutr. pp. 254-261. Clermont-Ferrand FR.

MEHREZ, A.Z. and E.R. ØRSKOV. 1979. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agr. Sci. (Camb.). 88:645.

MURPHY, J.J. and J.J. KENNELLY. 1986. Effect of level of undegradable protein in the diet on the performance of dairy cows in early lactation. J. Dairy Sci. 69 (Suppl. 1): 218.

MURPHY, J.J., J.J. KENNELLY and G. DE BOER. 1986. Effect of source and level of dietary crude protein on milk production and rumen fermentation in Holstein cows. J. Dairy Sci. 69 (Suppl. 1): 196.

ØRSKOV, E.R. 1982. Protein Nutrition in Ruminants. Academic Press Inc., London, U.K.

ØRSKOV, E.R. and I. McDONALD. 1979. The estimation of protein degradability in the rumen from incubation measurments weighted according to rate of passage. J. Agr. Sci. (Camb.) 92:499.

SETALA, J. 1983. The nylon bag technique in the determination of ruminal feed protein degradation. J. Sci. Agr. Soc. Finland. 55: 1.

STEELE, R.G.D. and J.H. TORRIE. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

STERN, M.D. and L.D. SATTER. 1984. Evaluation of nitrogen solubility and the dacron bag technique for estimating protein degradation in the rumen. J. Anim. Sci. 58:714.

STOCK, R., N. MERCHEN, T. KLOPFENSTEIN and M. POOS. 1981. Feeding value of slowly degraded proteins. J. Anim. Sci. 53:1109.

VAN SOEST, P.J., C.J. SNIFFEN, D.R. MERTENS, D.G. FOX, P.H. ROBINSON and U. KRISHNAMOORTHY. 1982. A net protein system for cattle: The rumen submodel for nitrogen. In: F. N. Owens (Ed.) Protein Requirements for Cattle. pp 265-279. Oklahoma State Univ., Stillwater.

VIK-MO, L. and J.E. LINDBERG. 1985. In sacco degradability of protein (N) and dry matter (DM) in samples of individual feeds or combinations; tested with diets medium or high in protein. Acta Agr. Scand. 35:117.

WEAKLEY, D.C., M.D. STERN and L.D. SATTER. 1983. Factors affecting disappearance of feedstuffs from bags suspended in the rumen. J. Anim. Sci. 56:493.

ZINN, R.A., L.S. BULL and R.W. HEMKEN. 1981. Degradation of supplemental proteins in the rumen. J. Anim. Sci. 52:857.

III. INFLUENCE OF SUPPLEMENTAL PROTEIN SOURCE AND PROTEIN CONCENTRATION ON RUMINAL AND INTESTINAL DIGESTION [3].

A. INTRODUCTION

Recognition of inherent deficiencies in protein systems based on crude protein (CP) and digestible CP (DCP), specifically that diets containing the same amount of CP or DCP will not necessarily support the same level of productivity, has resulted in the development of several new ruminant protein feeding systems (Van Soest et al. 1982; ARC, 1985; NRC 1985). Formulation of diets for ruminants using the new systems dictates that sufficient rumen degradable protein (RDP) is available to optimise microbial fermentation in the rumen, and that additional protein is supplied in the form of rumen undegradable protein (UDP) in situations where microbial protein synthesis is not sufficient to meet the protein requirements of the animal. Effective diet formulation then, is highly dependent on accurate estimation of UDP, microbial nitrogen (N) synthesis, and subsequent total protein supply to, and digestion and absorption in, the small intestine.

Rumen protein degradability values are commonly determined either by the in situ nylon bag technique (Ørskov and McDonald 1979) or from in vivo measurements using duodenally cannulated animals. Estimates of degradability obtained using the in situ nylon bag technique can be influenced by factors such as pore size, surface area, incubation time and mesh size of grinder screen (Meyer and Mackie 1986; Setälä 1983), while in vivo estimates are subject to error from assumptions regarding the amount of endogenous N entering the small intestine (Egan et al. 1984), variability in particle marker techniques (Santos et al. 1984), and errors associated with unrepresentative duodenal samples (Robinson, personal communication). Although the correlation between in situ and in vivo values can be relatively poor, they both tend to rank protein sources in the same order (Lindberg 1983; Madsen and Hvelplund 1985).

[3]This chapter has been submitted for publication in J. Anim. Sci.

A variety of protein sources have been evaluated to determine their resistance to ruminal microbial attack and the resulting amount of protein available to, and digested in the small intestine (Zinn et al. 1981; Loerch et al. 1983a; Santos et al. 1984; Garret et al. 1987). Several factors can influence ruminal degradation rate and ruminal outflow of protein to the small intestine. Among these are hormonal influences (Gonzales et al. 1985), level of feed intake (Robinson et al. 1985; Madsen 1986), and frequency of feeding (Tamminga et al. 1979). Further, protein concentration has been shown to influence ruminal degradability of protein sources in some experiments (Vik-Mo and Lindberg 1985; Madsen 1986; Kirkpatrick and Kennelly 1987) but not in others (Murphy and Kennelly 1987).

Although meat and bone meal (MBM) is not a traditional ingredient in the diet of ruminant animals, it is of considerable interest because of its low ruminal degradability (10 to 30%) in vitro (ARC 1985) and in vivo (Zinn et al. 1981; Loerch et al. 1983b). However, growth trials with cattle and lambs (Stock et al. 1981) demonstrated that the quality of MBM can vary considerably. This may result from the current processing technique of (vat-drying) which involves heating via steel pipes, where prolonged exposure to high temperatures can occur. Research by Craig and Broderick (1984) suggests that the essential amino acid profile of MBM reaching the small intestine may actually be enhanced after microbial attack in the rumen due to a disproportionate release of nonessential amino acids during ruminal degradation. If properly processed, then, MBM can be a good source of UDP for dairy cattle.

The objectives of this study were to examine the effect of protein source, (canola meal, soybean meal and meat and bone meal) and protein system formulation (CP and/or UDP) on supply of dry matter (DM) and CP to the small intestine of dairy heifers.

B. MATERIALS AND METHODS

Six pregnant Holstein Freisian heifers (300 to 350 kg body weight) were assigned to six dietary treatments in a 6 x 6 Latin square. soft rumen cannula (Bar diamond Inc,. Parma, Idaho) and a T-type duodenal cannula (2.5-cm i.d.) in the proximal duodenum 5-10 cm

distal to the pylorus. Distance from the pylorus was confirmed via endoscopic examination. Experimental diets were based on chopped brome alfalfa hay and barley (control) supplemented with canola meal (CM), soybean meal (SBM) or meat and bone meal (MBM). Diets (Table III.1) were designed to compare these four protein sources in addition to CM and SBM at two dietary protein levels. The six concentrate mixtures were: barley 14% CP (B14), barley/CM 16.5% CP (CM16.5), barley/SBM 16.5% CP (SBM16.5), barley/MBM 16.5% CP (MBM16.5), barley/CM 19% CP (CM19) and barley/SBM 19% CP (SBM19). Diets CM16.5, SBM16.5 and MBM16.5 were equivalent on a CP basis, whereas on a calculated UDP equivalent basis (ARC 1985) MBM16.5, CM19 and SBM19 were similar. Heifers were fed a 50:50 hay concentrate mix by automated feeders in 12 equal amounts each day, at 2-h intervals.

Digesta flow rate was calculated using dysprosium (Dy) and cobalt (Co) as Cobalt-ethylenediamenetetraacetic acid (Co-EDTA). The preparation of markers was as outlined by Kennelly et al. (1980) and Kennelly et al. (1982). Markers were incorporated into 5 kg subsamples of the total diet. Five grams of marker mix was included with each of the twelve daily feeds in order to minimize variation in marker concentration.

Animals were confined to metabolic crates during the 7 d adjustment, 7 d total fecal collection and 3 d digesta collection period with a minimum of a 7 d rest period in bedded pens, between each Latin square period. The 12 times daily feeding protocol (including marker mix) was maintained during the rest period in pens. Animals had continuous access to water and salt.

Fecal samples were collected once daily in plastic pans, weighed and an aliquort of total feces was dried in a forced-air oven at 65°C for 48 h. Upon completion of each period, samples were pooled on a per animal basis and stored for later analysis. Approximately 200 mL rumen, and 200 mL duodenal digesta were obtained through the cannula 3 x/d (30, 60 and 90 min after feeding) on each of the last 3 d of each experimental period. Rumen samples were strained through 4-layers of cheesecloth, pH was measured using a general-purpose

electrode, and samples were immediately stored at -20 C. Portions of digesta samples taken from the duodenum were strained through nylon gauze, with aperatures about 100 x 200 μm, as outlined by Faichney (1975) and filtrate was frozen. Both total digesta and filtrate were lysophilized prior to determination of Dy and cobalt (Co) concentration by neutron activation analysis. The sample preparation and analytical conditions were the same as those reported by Kennelly et al (1980; 1982). Ammonia in rumen and duodenal samples were measured using an ammonia electrode (HNU Systems, Inc., Newton, MA). Dry matter, organic matter (OM), and total nitrogen (N) were determined as outlined by AOAC (1980). Digesta flow rates were calculated from the concentrations of Dy and Co in diets, digesta and digesta filtrate using dual marker techniques (Faichney 1975). Nucleic acids were extracted by the method of Zinn and Owen (1986) for microbial N determination.

All data collected were analysed using analyses of variance procedures with treatment, animal and period as factors. Treatment means were compared using the Student-Newman-Keuls test when treatment effect was significant (Steel and Torrie 1980).

C. RESULTS AND DISCUSSION

All diets were readily consumed and feed refusal was rare. Zinn et al. (1981) reported reduced feed intake in animals fed diets containing MBM from a variety of sources and processing conditions. The processing method used by Zinn et al. (1981) was a vat-drying system where contact of MBM with heated steel pipes can cause heat damage which may reduce palatability. In contrast, the MBM used in this experiment was processed by boiling in oil. This process results in more uniform heat distribution such that the MBM product is less likely to suffer heat damage.

Diets were formulated using barley, a readily fermentable carbohydrate source, so that energy availability was unlikely to be a limiting factor for ruminal microbial growth. Total volatile fatty acid (VFA) concentrations were not affected by protein source or concentration (Table III.2), although diets containing SBM tended to have the highest total VFA

concentrations. Acetic acid levels were significantly lower ($P<.05$) for MBM16.5 and the CM diets (CM16.5, CM19). Animals fed MBM16.5 had significantly lower ($P<.05$) propionate concentrations than SBM16.5 and SBM19. Isobutyric concentrations were highest ($P<.05$) for SBM based diets and CM19, and lowest for B14. Animals fed the SBM diets had higher ($P<.05$) isovaleric concentrations than all other diets. Santos et al. (1984) reported similar results for diets supplemented with SBM. Significant differences in ruminal branched chain VFA concentrations for the SBM diets suggest an enhanced breakdown of valine and leucine.

Ruminal ammonia (NH_3) levels were affected by both level and source of protein in the diet (Table III.2). Animals fed diet B14 had significantly ($P<.05$) lower NH_3 levels than those fed other diets, while diets containing 16.5% CP (CM16.5, SBM16.5 and MBM16.5) had similar levels. Ruminal NH_3 levels for both the CM19 and SBM19 diets were significantly ($P<.05$) different from each other and from all other diets. Recent work by Odle and Schaeffer (1987) suggests that a portion of the variation in estimates for optimal NH_3 concentrations (Satter and Slyter (1974) - 3.5 mmol NH_3-N/l vs Mehrez et al. (1977) - 14 mmol NH_3-N/l) may be due to the type of degradable substrate fed. Ammonia concentration required to achieve maximal rates of ruminal degradation of barley DM were 7.8 mmol/L in contrast to 4.4 mmol/L for corn. Further, Smith and Oldham (1983) have suggested that bacteria may utilize different pathways of ammonia assimilation ie. passive diffusion vs active transport, to maintain cellular ammonia concentrations. Although NH_3 levels varied between diets, NH_3 concentrations were in the optimal range for all diets (Odle and Schaeffer 1987). Protein level and source had no effect on rumen pH values which were optimal (6.1 to 6.3) for rumen proteolysis and deamination according to Lewis and Emery (1962).

Duodenal pH values ranged from 2.5 to 2.8 and were within the range expected for samples taken from the proximal duodenum (Czerkawski 1986). Endoscopic examination revealed that cannulae were located within 10 cm of the pyloric sphincter in 5 of the 6 animals. In the sixth animal the cannula was located approximately 18 - 20 cm from the pyloric spincter. Periodic endoscopic examination of the intestine indicated that the intestine

surrounding the cannula was healthy with no visible pouching.

Feed DM intake was maintained at a constant level over the course of the experiment. However, due to substantial increases in body weight during the course of the experiment DM intake, as a % of body weight, decreased from approximately 2.4% at the beginning to 1.9% at the end of the experiment. Total duodenal DM flows for heifers (Table III.3) fed B14 were lower than for all other diets, with the exception of SBM16.5. Ruminal digestibility of diet DM, intestinal DM digestiblity and apparent fecal DM digestibility were not ($P>.05$) influenced by diet.

Organic matter intake of MBM16.5 was significantly ($P<.05$) lower than all other diets due to the lower OM of the MBM product. Protein concentration and source influenced ($P<.05$) OM digestibility (Table III.4). Ruminal digestibility of OM was highest ($P<.05$) for B14 and lowest ($P<.05$) for MBM16.5. These results are similar to comparisons made by Zinn et al. (1981) between SBM and MBM diets. All other diets had similar ruminal OM digestibilities. Intestinal digestibility of diet OM was not ($P>.05$) affected by protein level or source. Total tract OM digestibility of MBM16.5 was significantly ($P<.05$) lower than SBM19 while all other diets did not differ.

Total N flow to the duodenum was affected by protein concentration and source (Table III.5) with values for B14 being lower ($P<.05$) than that for all other diets. Animals fed SBM19 had highest duodenal total N flows with differences being higher ($P<.05$) than observed for B14, CM16.5 and SBM16.5. Values for MBM16.5 were similar to CM19 but differed ($P<.05$) from B14. Results suggest that the formulation of these diets (MBM16.5, CM19 and SBM19) on a UDP equivalent basis was effective in promoting similar flows of rumen escape N to the small intestine.

Flow of non-ammonia-nitrogen (NAN) to the duodenum (Table III.5) was lower than N intake for all diets. It has been observed (Loerch et al. 1983b; Santos et al. 1984; Garret et al. 1987) that NAN flow to the duodenum from diets containing less than 17% CP (DM basis) are often, though not always, greater than N intakes. It is suggested that this

may be due to the efficient utilization of dietary N and recycled urea for microbial growth (McAllan et al. 1986) Duodenal N flow for all diets was slightly less than N intake. Zinn et al. (1981) observed that in diets containing greater than 17% CP duodenal N flows were less than N intake. Duodenal NH_3 flow for SBM19 was greater ($P<.05$) than for CM19 while all other diets had signifigantly lower flow rates than CM19. These results reflect rumen ammonia levels for the various diets. Microbial N flow to the duodenum (Table III.5) was not ($P>.05$) affected by diet, protein source or protein level. Increasing total dietary-N, assuming energy is not a limiting factor, should increase microbial protein synthesis. In this experiment the efficiency of microbial N conversion decreased as protein level in the diet increased.

Estimates of dietary escape N (Table III.5) as a percent of intake were determined as the difference between duodenal NAN flow and microbial N flow. The resulting estimates are for the entire diet, as opposed to individual feedstuffs, and as corrections for endogenous protein were not made, final measurements represent apparent degradation or apparent N escape. Values for escape N ranged from 29.1 to 40.6%. The MBM16.5 diet was most resistant to rumen degradation with a higher proportion of N reaching the small intestine than the B14, CM16.5 and SBM16.5 diets. Values for escape N obtained for the MBM16.5 diet are similar to those reported by Zinn et al. (1981), for steers at similar levels of intake while values for the CM and SBM diets were similar to those reported by Ha and Kennelly (1984). Rumen undegradable protein values obtained for these diets using the in situ nylon bag technique (Kirkpatrick and Kennelly 1987) were 29.1, 28.6, 32.3, 35.0, 28.0, and 27.2 for B14, CM16.5, SBM16.5, MBM16.5, CM19 and SBM19, respectively. Both techniques ranked the MBM16.5 diet as least degradable in the rumen and highest in percentage of escape N. However, in situ estimates showed increased ($P<.05$) N escape as protein content in the diet increased while the reverse was true for the in vivo technique. Madsen, (1985) comparing two levels of SBM intake observed a decline in percent escape N with increasing dietary N concentrations for both in situ and in vivo techniques. Results for CM at two dietary CP levels were similar for

both techniques. The ranking for the basal diet was higher using the in vivo technique. Estimates of actual feed N escape may be influenced by level of feed intake and feeding frequency. Tamminga et al. (1979) observed that in animals fed twice daily, degradation of dietary protein was lower at higher levels of feed intake. This was attributed to an increased rate of passage through the forestomachs. Further, the efficiency of microbial protein synthesis was not affected by level of feed intake. Madsen (1985) evaluated SBM, cotton seed meal, grass pellets and fish meal at two levels of intake. While degradability of SBM was lower at higher levels of intake, the opposite was true for all other supplements. Tamminga (1981) reported that frequent feeding (6x/d vs 2x/d) decreased the proportion of feed N escaping degradation in the rumen. However this may be offset by a more efficient microbial protein synthesis in the rumen.

Microbial N synthesized/kg OM apparently digested in the rumen ranged from 20.3 to 30.7. These values are similar to those obtained by Veira et al. (1980) and Zinn et al. (1981) for cattle fed hay and concentrate diets. Garret et al. (1987) fed isonitrogenous straw diets with low rumen degradability to steers. Their results suggest that while feeding of highly resistant dietary N sources will increase escape of dietary N, microbial growth may be depressed, due to reduced N availability in the rumen. The MBM supplement used in this experiment may also have this effect under feeding conditions where RDP is in limited supply. However, on the basis of ARC (1985) requirements RDP was not limiting.

The nucleic acid:total N ratio technique (Zinn and Owens 1986) was used to estimate microbial N. This technique takes into account protozoal N which can be considerable under some feeding conditions (Harrison et al. 1979). Further, since the nucleic acid N:total N ratio technique includes both RNA and DNA it should be more constant than the RNA:N ratio technique.

Apparent ruminal digestibility of diet N tended to be influenced by both protein concentration and source. Nitrogen digestibility in the rumen decreased as protein level increased for the two SBM diets (SBM16.5 and SBM19) while the CM diets were unaffected.

Further the apparent ruminal digestibility of diet N for the MBM16.5 diet tended to be lower than the B14 diet and both 16.5% CP diets (CM16.5 and SBM16.5). These results are in agreement with Garret et al. (1987) who found that ruminal digestibility of diet N tended to decrease as ruminal degradability of the protein supplement increased.

Apparent intestinal N digestibility was affected by both protein concentration and source. Intestinal N disappearance was significantly ($P<.05$) greater for SBM19 than all other diets with the exception of MBM16.5. The apparent intestinal N digestibility for the MBM16.5 diet was significantly greater ($P<.05$) than B14 though it was similar to all others. The high intestinal N disappearance of the MBM16.5 diet reflects the absence of heat damage with the processing techniques used in the preparation of MBM.

Apparent fecal N digestibility was affected by both protein concentration and source, with diets B14 and MBM16.5 being significantly ($P<.05$) lower than all other diets. Both the 19% CP diets had significantly ($P<.05$) higher apparent fecal N digestibility than the 16.5% CP diets. Although ruminal digestibility of diet N was depressed for both the 19% CP diets and for MBM16.5, this depression was compensated for by enhanced intestinal digestibility.

A comparison based on predictive (ARC 1985; NRC, 1985) estimates and actual calculation (in situ and in vivo) of dietary RDP and UDP are presented in Table III.6. There are several potential reasons for the disagreement between in situ and in vivo estimates. In situ degradability estimates are influenced by diet, protein source and energy level (Loerch et al. 1983a; Madsen and Hvelplund 1986) and pH (Loerch et al. 1983a). Further, Madsen and Hvelplund (1986) found the differences were confounded for vegetable protein sources but not for fish meal protein sources. Comparison of in situ vs in vivo estimates of UDP (Table III.6) indicate that the in vivo technique resulted in a higher estimate for the vegetable protein diets and a lower estimate for the MBM diet. Theoretically, the new protein feeding systems provide a more accurate prediction of the protein supply to the small intestine, and thus improved prediction of animal performance. However, actual performance data which supports the UDP and RDP recommendations of the various feeding systems is lacking. In

general high producing animals respond to increased levels of UDP (Murphy and Kennelly 1987) in the diet, however the absolute levels which are required for specific production levels is far from being clearly defined.

In conclusion, the results suggest that when diets are formulated on the basis of RDP and UDP the prediction of animal performance is likely to be more accurate than when diets are formulated on the basis of CP. Further, concentrations of RDP and UDP provide a more accurate description of protein quality of feedstuffs than CP or DCP.

Table III.1 Formulation and Composition of Experimental Diets (%) Dry Matter Basis

Item	Diets[a]					
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19
Ingredients						
Alfalfa-brome hay	50.0	50.0	50.0	50.0	50.0	50.0
Barley (B)	46.3	34.3	36.8	36.8	25.0	31.0
Canola meal (CM)		12.0			21.3	
Soybean meal (SBM)			9.5			15.3
Meat and bone meal (MBM)				9.5		
Molasses	1.5	1.5	1.5	1.5	1.5	1.5
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0
TM salt[b]	0.25	0.25	0.25	0.25	0.25	0.25
Chemical analyses						
Crude protein	13.7	16.2	16.5	16.6	19.0	19.4
Acid detergent fiber	26.4	27.0	24.5	24.9	27.2	26.4
Calcium	0.86	0.93	0.96	1.77	1.14	1.01
Phosphorus	0.56	0.63	0.68	0.92	0.87	0.83
Ash	6.1	6.4	6.1	7.9	7.6	7.0

[a] All diets were fortified on a per kg basis with Vitamin A palmitate 4000 IU; Vitamin D 460 IU; Vitamin D, 123.7 IU; Vitamin E 67 IU.
[b] Trace mineral salt contained - Se 25 ppm, Co 40 ppm, Mg 0.35%, Cu 0.25%, I 0.01%, Zn 0.75%.

Table III.2 Effect of Dietary Protein Source and Concentration on Ruminal Volatile Fatty Acid Concentration and Proportion, Ruminal PH and Ammonia (NH_3) Concentration[a]

Item	Dietary treatment and concentrate type[b]						
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[c]
Total VFA, (m M)	94.9	96.2	99.0	90.1	91.5	100.3	3.61
Molar proportions, (%)							
Acetic acid (C_2)	66.6^{d}	62.5^{e}	65.9^{d}	62.4^{e}	62.6^{e}	68.0^{d}	0.71
Propionic acid (C_3)	$14.5^{e,f,g}$	$13.2^{e,f}$	16.2^{d}	12.8^{f}	$14.4^{e,f,g}$	$15.1^{d,e}$	0.53
Isobutyric	0.75^{d}	$0.95^{e,f}$	$1.08^{g,h}$	0.91^{e}	$1.06^{g,h}$	1.16^{g}	0.04
Butyric acid (C_4)	$12.2^{d,e}$	$12.0^{d,e}$	12.7^{d}	$11.2^{e,f}$	10.5^{f}	12.7^{d}	0.36
Isovaleric	1.2^{d}	1.2^{d}	1.5^{e}	1.3^{d}	1.3^{d}	1.7^{e}	0.06
Valeric acid	1.3^{d}	$1.4^{d,e}$	$1.5^{d,e}$	$1.4^{d,e}$	$1.5^{d,e}$	1.6^{e}	0.06
$C_3+C_4:C_2$	5.7	5.7	5.1	5.7	5.1	5.3	0.23
C_3/C_2 ratio	4.8^{d}	4.7^{d}	4.2^{e}	4.8^{d}	$4.4^{d,e}$	$4.5^{d,e}$	0.01
Rumen pH	6.2	6.2	6.2	6.1	6.2	6.3	0.14
Rumen NH_3, mmol/l	8.8^{d}	10.9^{e}	11.2^{e}	11.2^{e}	13.2^{f}	17.7^{g}	0.17

[a] Each value represents the mean of six observations.

[b] See Table IV.1 for details.

[c] Standard error of the mean.

[d,e,f,g] Means in the same row with a different letter in their superscripts differ ($P<.05$).

Table III.3 Effect of Dietary Protein Concentration and Source on Ruminal, Intestinal and Total Tract Apparent Digestion (%) of Dry Matter (DM)[a]

Item	Dietary treatment and concentrate type[b]						
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[c]
Intake, kg DM/d	8.5	8.5	8.5	8.5	8.5	8.5	
Flow to duodenum, kg DM/d	3.80[f]	4.39[g]	4.14[fg]	4.41[g]	4.42[g]	4.33[g]	.20
Ruminal DM digestibility, %	55.86	48.96	51.76	48.81	48.54	49.65	2.28
Fecal output, kg DM/d	2.93[d]	3.17[de]	3.24[e]	3.29[e]	3.23[e]	2.90[d]	.10
Intestinal DM digestibility, %	21.7	27.3	21.9	24.7	24.6	32.0	4.4
Total fecal DM digestibility, %	68.12[d]	65.73[de]	64.91[e]	64.37[e]	64.98[e]	68.26[d]	.96

[a]Each value represents the mean of six observations.
[b]See Table IV.1 for details.
[c]Standard error of the mean.
[d,e]Means in the same row without a common letter in their superscripts differ ($P<.05$).
[f,g]Means in the same row without a common letter in their superscripts differ ($P<.10$).

Table III.4 Effect of Supplemental Protein Concentration and Source on Ruminal, Intestinal and Total Tract Apparent Digestion (%) of Organic Matter (OM)[a]

Item	Dietary treatment and concentrate type[b]						
	BI4	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[c]
OM Intake, kg/d	8.02[d]	7.95[d]	8.00[d]	7.45[e]	7.94[d]	8.09[d]	.04
OM Flow to duodenum, kg/d	3.48	4.00	3.84	4.05	3.97	3.88	.18
Ruminal OM digestibility, %	56.7[d]	49.8[e]	51.9[e]	45.5[f]	50.1[e]	52.2[e]	.91
Fecal OM output, kg/d	2.20[d]	2.38[e]	2.48[e]	2.34[e]	2.42[e]	2.68[e]	.23
Intestinal OM digestibility, %	35.46	40.19	34.79	41.69	36.80	42.80	3.51
Fecal OM digestibility	72.1[de]	69.9[de]	69.0[de]	68.7[e]	69.7[de]	72.8[d]	1.19

[a]Each value represents the mean of six observations.
[b]See Table IV.1 for details.
[c]Standard error of the mean.
[d,e,f]Means in the same row without a common letter in their superscript differ ($P<.05$)

Table III.5 Effect of Supplemental Protein Concentration and Source on Ruminal, Intestinal and Total Tract Apparent Digestion (%) of Nitrogen (N)[a]

Item	Dietary treatment and concentrate type[b]						
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE[c]
Intake, g/d	188.5[e]	223.0[f]	227.2[f]	228.4[f]	261.5[g]	267.5[g]	1.38
Flow to duodenum, g/d							
Total N	172.9[e]	191.3[f]	202.5[f]	224.6[fg]	225.6[fg]	241.1[g]	11.30
Ammonia N	16.9[e]	27.0[f]	29.8[f]	28.8[f]	33.9[g]	43.7[h]	.16
Microbial N	91.2	97.9	105.5	102.9	110.6	94.9	7.15
Diet N	64.9[e]	66.4[e]	67.2[e]	92.9[ef]	81.1[ef]	103.4[f]	7.97
Ruminal N digestibility, %[d]	56.8[ij]	58.8[i]	57.6[i]	46.8[jk]	55.7[ijk]	45.8[k]	4.03
Microbial N synthesis, g/kg OM apparently digested	20.3	24.8	26.3	30.6	30.7	22.4	3.22
Escape of diet N, % of intake[d]	34.2[i]	29.1[i]	29.3[i]	40.6[j]	31.2[ij]	38.3[ij]	3.83
Intestinal N digestibility, %	63.5[e]	66.2[ef]	66.3[ef]	70.9[fg]	67.2[ef]	74.6[g]	2.73
Fecal N output, g/d	60.6[e]	63.9[e]	66.4[ef]	71.8[f]	64.9[e]	60.3[e]	1.99
Fecal N digestibility, %	67.8[e]	71.3[f]	70.8[f]	68.5[e]	75.1[g]	77.4[h]	.85

[a]Each value represents the mean of six observations.
[b]See Table IV.1 for details.
[c]Standard error of the mean.
[d]Corrected for bacterial and protozoal N but not endogenous N.
[e,f,g,h]Means in the same row without a common letter in their superscript differ ($P<.05$).
[i,f,k]Means in the same row without a common letter in their superscript differ ($P<.10$).

Table III.6 Ruminal Degraded Protein (RDP) %, Ruminal Undegraded Protein (UDP) % Calculated on the Basis of In Situ and In Vivo Data Compared to ARC and NRC Estimates of Requirements

		RDP				UDP			
Diets[a]	Ingredients	ARC[b]	NRC[c]	IN SITU[d]	IN VIVO	ARC	NRC	IN SITU	IN VIVO
B14	Alfalfa/Barley	72.2	75.2	70.8	65.7	27.7	24.8	29.2	34.3
CM16.5	Alfalfa/Barley/ Canola meal	72.2	74.1	71.6	71.0	27.8	25.9	28.4	29.6
SBM16.5	Alfalfa/Barley/ Soybean meal	67.9	72.7	67.9	70.9	32.1	27.3	32.1	29.1
MBM16.5	Alfalfa/Barley/ Meat and bone meal	61.4	62.0	65.1	59.6	38.5	37.9	34.9	40.3
CM19	Alfalfa/Barley/ Canola meal	73.7	74.7	72.1	68.9	26.3	25.2	27.9	31.0
SBM19	Alfalfa/Barley/ Soybean meal	67.5	73.2	72.7	61.8	32.0	26.8	27.3	38.1

[a] See Table 1 for details
[b] ARC (1985)
[c] NRC (1985)
[d] Kirkpatrick, B.K. and J.J. Kennelly 1987.

D. BIBLIOGRAPHY

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). 1980. Official method of analysis. 12th ed. AOAC, Washington, D.C.

AGRICULTURAL RESEARCH COUNCIL (ARC). 1985. The Nutrient Requirements of Ruminant livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Farnham Royal, England.

CRAIG, W.M. and G.A. BRODERICK. 1984. Amino acids released during protein degradation by rumen microbes. J. Anim. Sci. 58:436.

CZERKAWSKI, J.W. 1986. Rumen Studies. Pergamon Press Ind. New York.

EGAN, A.R., K. BODA and J. VARADY. 1984. Regulation of nitrogen metabolism and recycling. In: Control of Digestion and Metabolism in Ruminants. L.P. Milligan, W.L. Grovum and A. Dobson eds., p. 386-401. Prentice-Hall. Englewood Cliffs, New Jersey.

FAICHNEY, G.J. 1975. The use of markers to partition digestion within the gastro-intestinal track of ruminants. In Digestion and Metabolism in the Ruminant. I.W. McDonald and A.C.I. Warner, eds. pp. 277-291. University of New England, Sidney, Australia.

GARRETT, J.E., R.D. GOODRICH, J.C. MEISKE AND M.D. STERN. 1987. Influence of supplemental nitrogen source on digestion of nitrogen, dry matter and organic matter and on in vivo rate of ruminal protein degradation. J. Anim Sci. 64:1801.

GONZALEZ, J.S., J.J. ROBINSON and C. FRASER. 1985. The effect of physiological state on digestion in the ewe and its influence on the quantity of protein reaching the abomasum. Livestock Prod. Sci. 12:59.

HA, J.K. and J.J. KENNELLY. 1984. In situ dry matter and protein degradation of various protein sources in dairy cattle. Can. J. Anim Sci. 64:443.

HARRISON, D.G., D.E. BEEVER and D.F. OSBOURN. 1979. The contribution of protozoa to the protein entering the duodenum of sheep. Brit. J. Nutr. 41:521.

KENNELLY, J.J., M.J. APPS, B.V. TURNER and F.X. AHERNE. 1980. Dysprosium, cerium and chromium marker determination by instrumental neutron activation analysis. Can. J. Anim. Sci. 60:749.

KENNELLY, J.J., B.V. TURNER and M.J. APPS. 1982. Determination of non-radioactive liquid phase markers, Co-EDTA and Cr-EDTA, by Neutron Activation Analysis. J. Sci. Food Agric. 33:1235.

KIRKPATRICK, B.K. and J.J. KENNELLY. 1987. In situ degradability of protein and dry matter from single protein sources and from a total diet. J. Anim. Sci. 65:557.

LEWIS, T.R. and R.S. EMERY. 1962. Intermediate products in the catabolism of amino acids by rumen organisms. J. Dairy Sci. 45:1363.

LINDBERG, J.E. 1983. Factors affecting predictions of rumen degradability using the nylon bag (in sacco) technique and a comparison between in vivo and in sacco degradability measurements. Report 59 (Theses). Swedish Univ. Agr. Sci., Dept. Anim. Nutr.

LOERCH, S.C., L.L. BERGER, S.D. PLEGGE, D. GIANOLA and J.C. FAHEY JR. 1983a. Effect of dietary protein source and energy level on in situ nitrogen disappearance of various protein sources. J. Anim. Sci. 57:206.

LOERCH, S.C., L.L. BERGER, S.D. PLEGGE and J.C. FAHEY JR. 1983b. Digestibility and rumen escape of soybean meal, blood meal, meat and bone meal and dehydrated alfalfa nitrogen. J. Anim. Sci. 57:1037.

MADSEN, J. 1986. Influence of feeding level on digestion and protein passage to the duodenum in cows fed high concentrate diets. Acta Agric. Scand. 36:275.

MADSEN, J. and T. HVELPLUND. 1985. Protein degradation in the rumen. A comparison between in vivo, nylon bag, in vitro and buffer measurements. J. Acta Agric. Scand. 25:103.

McALLAN, A.B., SIDDONS R.C. and BEEVER D.E. 1986. The efficiency of conversion of degraded nitrogen to microbial nitrogen in the rumen of sheep and cattle. J. Agric. Sci. Camb.

MEHREZ, A.Z., E.R. ØRSKOV and I. MCDONALD. 1977. Rate of rumen fermentation in relation to ammonia concentration. Brit. J. Nutr. 38:437.

MEYER, J.H.F. and R.I. MACKIE. 1986. Microbiological evaluation of the intraruminal In Sacculus digestion technique. Appl. Envir. Microbiology. 51:622.

MURPHY, J.J. and J.J. KENNELLY. 1987. Effect of protein concentration and protein source on the degradability of dry matter and protein in situ. J. Dairy Sci. 70:1841.

NRC. 1985. Ruminant Nitrogen Usage. National Research Council - National Academy of Sciences, Washington, D.C.

ODLE, J. and D.M. SHAEFER. 1987. Influence of rumen ammonia concentration on the rumen degradation rates of barley and maize. Brit. J. Nutr. 57:127.

ØRSKOV, E.R. and I. MCDONALD. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. Camb. 92:499.

ROBINSON, P.H., C.J. SNIFFEN and P.J. VAN SOEST. 1985. Influence of level of feed intake on digestion and bacterial yield in the forestomachs of dairy cattle. Can. J. Anim. Sci. 65:437.

SANTOS, K.A., M.D. STERN and L.D. SATTER. (1984). Protein degradation in the rumen and amino acid absorbtion in the small intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci., 58:244.

SATTER, L.D. and L.L. SLYTER. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Brit. J. Nutr. 32:119.

SETALA, J. 1983. The nylon bag technique in the determination of ruminal feed protein degradation. J. Sci. Agr. Soc. Finland 55:1.

SMITH, R.H. and J.D. OLDHAM. 1983. Rumen metabolism and recent developments. In: International Atomic Energy Agency (Ed.), Nuclear Techniques for Assessing and Improving Ruminant Feeds. pp. 1-35. Vienna, Austria.

STEEL, R.G.D. and J.W. TORRIE. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Company. New York.

STOCK, R., N. MERCHEN, T. KLOPFENSTEIN and M. POOS. 1981. Feeding value of slowly degraded proteins. J. Anim. Sci. 53:1109.

TAMMINGA, S. 1981. Nitrogen and amino acid metabolism in dairy cows. Ph.D. Thesis, Agric. Univ., Wageningen, The Netherlands.

TAMMINGA, S., C.J. VAN DER KOELEN and A.M. VAN VUREN. 1979. Effect of the level of feed intake on nitrogen entering the small intestine of dairy cows. Livestock Production Science. 6:255.

VAN SOEST, P.J., C.J. SNIFFEN, D.R. MERTENS, D.G. FOX, P.H. ROBINSON and U. KRISHNAMOORTHY. 1982. A net protein system for cattle: The rumen submodel for nitrogen. In: F.N. Owens (Ed.). Protein Requirements for Cattle. pp. 265. Oklahoma State Univ., Stillwater.

VEIRA, D.E., G.K. MACLEOD, J.H. BURTON and J.B. STONE. 1980. Nutrition of the weaned holstein calf. I. Effect of dietary protein level on rumen metabolism. J. Anim. Sci. 50:937.

VIK-MO, L. and J.E. LINDBERG. 1985. In sacco degradability of protein (N) and dry matter (DM) in samples of individual feeds or combinations; tested with diets medium or high in protein. Acta Agr. Scand. 35:117.

ZINN, R.A., L.S. BULL and R.W. HEMKEN. 1981. Degradation of supplemental proteins in the rumen. J. Anim. Sci. 52:857.

ZINN, R.A. and F.N. OWENS. 1986. Rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. J. Anim. Sci. 66:157.

IV. THE MOBILE NYLON BAG TECHNIQUE AS A PREDICTOR OF THE NUTRITIVE VALUE OF FEEDSTUFFS FOR DAIRY CATTLE [4].

A. INTRODUCTION

The concentration of rumen degradable protein (RDP) and of undegraded dietary protein (UDP) in ruminant feedstuffs more accurately reflects protein quality in ruminant feedstuffs than does crude protein (CP) content (Agricultural Research Council (ARC) 1985). In high producing dairy cows, microbial protein synthesis from RDP is generally not sufficient to meet the protein needs of these animals. Under these circumstances, microbial protein supply to the intestine must be augmented by substantial quantities of UDP. Extensive research has been completed on protein degradability in the rumen (ARC 1985; National Research Council (NRC) 1985); however, there are very few reports (Rooke et al. 1981) on the digestion of the UDP fraction in the small intestine. Intestinal digestion of UDP is an essential prerequisite to the utilization of UDP by the animal. Protein which has been rendered unavailable in the rumen through heat damage or chemical treatment, and protein sources which tend to have naturally low availability (eg. feather meal) can be excellent sources of UDP. However, if the UDP is indigestible in the intestine it will not contribute to the protein needs of the animal. The research presented here, previously published in abstract form (Kirkpatrick and Kennelly 1984) describes a modified or mobile nylon bag (MNB) technique for estimating intestinal digestibility of UDP. The technique is a modification of that first described for use in swine by Sauer et al. (1983).

B. MATERIALS AND METHODS

Animals and feeding

Six pregnant Holstein heifers, 325 ± 25 kg body weight were assigned in a 6 x 6 Latin square design, to six dietary treatments. Each heifer was equipped with urine diversion

[4]This chapter has been conditionally accepted for publication in the Can. J. Anim. Sci.

harnesses and fitted with a 10-cm (i.d.) soft rumen cannula (Bar diamond Inc., Parma, Idaho) and a T-type duodenal cannula (2.5-cm i.d.) in the proximal duodenum 5-10 cm distal to the pylorus. Distance from the pylorus was confirmed via endoscopic examination. Further, periodic endoscopic examination of the intestine indicated that the intestine surrounding the cannula was healthy with no visible pouching thus ensuring that bags would not be held in the cannula opening. Experimental diets were based on chopped brome alfalfa hay and barley (control) supplemented with canola meal (CM), soybean meal (SBM) or meat and bone meal (MBM). Diets (Table IV.1) were designed to compare these four protein sources in addition to CM and SBM at two dietary protein levels. The six concentrate mixtures were: barley 14% CP (B14), barley/CM 16.5% CP (CM16.5), barley/SBM 16.5% CP (SBM16.5), barley/MBM 16.5% CP (MBM16.5), barley/CM 19% CP (CM19) and barley/SBM 19% CP (SBM19). Diets CM16.5, SBM16.5 and MBM16.5 were equivalent on a CP basis, whereas on a calculated UDP equivalent basis (ARC 1985) MBM16.5, CM19 and SBM19 were similar. Heifers were fed 8.5 kg of a complete diet consisting of a 50:50 hay concentrate mix by automated feeders, in 12 equal amounts each day, at 2 hr intervals.

All feeds were obtained commercially and ground though a 2-mm screen prior to being placed in nylon bags. The MBM product was commercially processed by vat drying (boiling in oil) at 250°C, rather than the more common process of heating in steel pipes. Nylon bag evaluation of protein sources (PS) and total diets (TD) was only carried out in animals fed that PS or TD. For example, CM PS was evaluated in animals fed CM16.5 and CM19.

Nylon Bags

Bags, measuring 3.5 x 5.5 cm, were made of nylon cloth (B and SH Thompson and Co. Ltd., Montreal) with a mesh size of 48μm (which was confirmed by microscopic examination). Approximately 2.0 g (air dry) of the PS or 1.5 g of the TD being evaluated, were placed in each nylon bag which was heat sealed (Audion Impulse Sealer, Audion Electro, Packing Aids Company, 469 Bryant Street, P.O. Box 7723, San Francisco, Ca, 94107). Bags were trimmed and the corners rounded, to avoid damage to the small intestine.

Rumen and pepsin HCl incubation

Ten bags per test feed per animal (10 samples for each of 6 animals = 60 bags per test feed) were attached to the inside of nylon stockings and incubated in the rumen for 15 h. Previous work by Kirkpatrick and Kennelly (1987) with the same diets determined that ruminal digestion of CP and DM from standard nylon bags was virtually complete after 15 h. Upon removal from the rumen, the bags were incubated in a pepsin HCl solution (1 g pepsin/L 0.1N-HCl), (Sauer et al. 1983) adjusted to pH 2, at 37.5°C for 3 h with constant stirring. After both rumen and pepsin HCl digestion one bag from each set was retained for DM and CP analysis.

Intestinal insertion and recovery of bags in the feces

After pepsin-HCl digestion, all bags were put on ice and kept refrigerated at 3°C. Bags (6 bags/test feed/animal) were introduced into the small intestine, via the duodenal cannula, at the rate of 2 per 2 h, using curved tissue forceps. Up to 14 bags per animal per day were inserted into the intestine. The bags were recovered from the feces, wiped clean with a tissue and dried in a forced air oven at 60°C for 48 h as described by Sauer et al. (1983).

The contents of each bag were subjected to Kjeldahl nitrogen (N) analysis (AOAC 1980, method no. 7.015). The percent disappearances of CP and DM were calculated from the proportion of the original weight remaining in the bags.

Data were subject to analysis of variance. Period effects were not significant ($P>0.05$); therefore, treatments were tested for significance against animal x test protein.

When F values were significant ($P<0.05$), treatment means were compared at a probability level of 0.05 using Student-Newman-Keuls test (Steele and Torrie 1980).

C. RESULTS AND DISCUSSION

The disappearance of CP from nylon bags after 15 h incubation in the rumen, 3 h digestion in pepsin HCl and recovery in the feces, for the PS and TD, are in Tables IV.2 and IV.3 respectively. Although this is a significantly higher weight to surface area ratio than is recommended (Setala 1983) for rumen incubation experiments, due to the high intestinal disappearance of sample material seen in swine experiments Sauer (personal communication) recommended a higher sample weight to bag size. Ruminal plus intestinal crude protein disappearance rates for PS were about 10 units higher than observed for TD. Disappearance values ranged from 79.1 to 92.9% for PS, whereas TD disappearance values ranged from 65.6 to 77.0.

The pH of abomasal contents in dairy cattle fed mixed diets is slightly greater than pH 2 (Church 1976). The principle function of pepsin and HCl in protein digestion is indirect, as together they expose peptide bonds within protein molecules. This improves access by pancreatic enzymes and results in more effective protein digestion. Hvelplund (1985) found significant changes in CP disappearance in the small intestine when formaldehyde treated SBM was incubated with abomasal contents of different pH. Crude protein disappearance of formaldehyde treated SBM was significantly lower than untreated SBM at all pH levels; CP disappearance decreased (0.75 to 0.54) as pH of abomasal contents increased from 2.21 to 3.22.

On the basis of intestinal CP disappearance data for protein sources in this experiment, pepsin-HCl incubation appears to have limited influence on the extent of CP disappearance. This was confirmed by de Boer et al, (1987) who found that high CP disappearance was achieved in the absence of pepsin-HCL.

The effect of the pepsin-HCl appeared to be minimal at the higher protein levels (CM19 and SBM19). Increased CP disappearance in samples pre-incubated in the rumen after pepsin-HCl incubation are likely due to the rinsing out of soluble CP. This is consistent with Cherian (1985) and Sauer et al. (1983) who found no immediate effects of pepsin-HCl incubation on various protein sources.

Dry matter disappearance values for protein sources (Table IV.4) were considerably less than those obtained for CP (Table IV.2). Dry matter disappearance values for TD (Table IV.5), were low relative to estimates obtained using total fecal collection. However, the relatively high correlation ($r = .86$) observed suggested that the technique could be used to rank feedstuffs. Subsequent experiments (de Boer et al. 1986a; de Boer et al. 1987) suggest that the relatively low DM disappearance values observed in this study, could be attributed to the methods used for suspension of nylon bags in the rumen (nylon stockings), cleaning rather than washing of bags, and sample volume to nylon bag surface area. Replacement of nylon stockings as a method of rumen containment with a polyester mesh bag (mesh size 3 mm), (de Boer et al. 1986a; de Boer et al. 1987), resulted in greater CP and DM disappearance in the rumen. In addition, washing of bags (de Boer et al. 1986a; de Boer et al. 1987), in contrast to removal of adhering particulate matter, is probably a critical factor and also resulted in achieving higher estimates of CP and DM disappearance.

A comparison between the MNB technique and the traditional total fecal collection method for the determination of CP digestibility is shown in Table IV.6. The MNB CP digestibility results are consistent with the total fecal collection technique ($r = .99$) though slightly lower. The regression equations using the MNBT to predict apparent CP digestibility and apparent DM digestibility are, $CP = 4.09 + .97(MNBT)$ and $DM = 10.77 + .86(MNBT)$, respectively. The reduced (mean = 1.9%) CP digestibilities obtained using the MNB technique may have been due to losses incurred during final recovery of material from the bags. This is consistent with results obtained by Sauer et al. (1983), who noted a slight underestimation of CP disappearance using a similar technique in pigs. The difficulty of recovering material from

bags has subsequently been overcome (de Boer et al. 1986; de Boer et al. 1987) by using Nytex material and a self-sealing procedure, such that the entire bag and contents is subject to CP analysis. The difficulties associated with achieving complete measurement of intake and fecal output with the conventional total collection method may also result in slight overestimation of digestibility.

The bag pore size of 48 μm was chosen because it allowed ruminal digestion and subsequent intestinal digestion, without the additional step of transferring the sample to smaller pore size bags. The pore size of the bags is of concern as it must be small enough to avoid losses of undigested particles yet, large enough to allow intestinal contents to flow freely through the bags. Cherian (1985) using pigs, compared intestinal disappearance of CP from 48, 20 and 10 μm pore size bags. Intestinal CP disappearance was more accurately determined using 48 μm pore size. The use of the MNB technique to determine "true" CP digestibility, where bags are rinsed upon recovery from the feces (Hvelplund 1985), may necesitate the use of smaller pore size material to eliminate the flushing of undigested nitrogenous material associated with DM out of the bag.

The average residence time of bags (n=145) in the intestine was 15.8 h (6 h minimum and 32.0 h maximum). These times are based on observations made every 2 h. There was a marked variation in passage time; however, this did not appear to affect CP disappearance. Hvelplund (1985) and Voight et al. (1985) have reported similar results.

There is concern that significant microbial contamination of bags may occur in the large intestine, particularly with high fibre diets. Hvelplund (1985), using N-free cellulose, determined that the digestibility of the cellulose between the terminal ileum and feces was 3-5% indicating some microbial activity. However the CP content of the residue was only 0.39 mg/g suggesting that residual microbial contamination is unlikely to have a significant influence on digestibility estimates.

There are few estimates of UDP digestibility in ruminants. Rooke et al. (1981) determined that the UDP fraction from SBM had a higher true digestibility than nonruminally

digested SBM in rats. Hvelplund (1985), using sheep, estimated the true digestibilities of SBM and CM to be 97% and 76%, respectively. The low CP digestibility of CM was attributed to high levels of cell wall bound nitrogen. Voight et al. (1985) determined the true CP digestibility of SBM, CM and barley in dairy cattle to be 97.2, 91.8 and 89.6%, respectively. De Boer et al. (1986) estimated the true digestibility of MBM CP to be 80.1% after 24 h incubation in the rumen. The results of the present study for CP digestibilities are in good agreement with the above research.

The CP disappearance values derived using the MNB technique, for the various TDs tested, showed significant differences between diets. These results are in good agreement with the total fecal collection method (Table IV.6), indicating that the technique is valuable as a rapid method for the estimations of apparent CP digestibility. In addition, the MNB technique can be used to obtain estimates of intestinal digestion of CP for individual components of the diet. Finally, the use of the technique to determine the intestinal availability of UDP is particularly attractive.

Table IV.1 Formulation and Composition of Experimental Diets (% dry matter basis).

Ingredients	Diets†					
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19
Alfalfa-brome hay	50.0	50.0	50.0	50.0	50.0	50.0
Barley (B)	46.3	34.3	36.8	36.8	25.0	31.0
Canola meal (CM)	—	12.0	—	—	21.3	—
Soybean meal (SBM)	—	—	9.5	—	—	15.3
Meat and bone meal (MBM)	—	—	—	9.5	—	—
Molasses	1.5	1.5	1.5	1.5	1.5	1.5
DiCalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0
TM Salt‡	0.25	0.25	0.25	0.25	0.25	0.25
Chemical Analyses:						
Crude protein	13.7	16.2	16.5	16.6	19.0	19.4
Acid detergent fibre	26.4	27.0	24.5	24.9	27.2	26.4
Calcium	0.86	0.93	0.96	1.77	1.14	1.01
Phosphorus	0.56	0.63	0.68	0.92	0.87	0.83
Ash	6.1	6.4	6.1	7.9	7.6	7.0

†All diets were fortified on a per kg basis with vitamin A palmitate 4000 IU; D 460 IU; D_3 123.7 IU; Vitamin 67 IU.

‡Trace mineral salt contains - Selenium 25 ppm, Cobalt .004%, Magnesium 0.35%, Copper 0.25%, Iodine 0.01% and Zinc 0.75%.

Table IV.2 Ruminal and intestinal crude protein disappearance (%) of protein source (PS) samples using the mobile nylon bag technique.[†]

Diet Fed[‡]	Incubated Sample[d]	Rumen n=6	Rumen plus Pepsin HCL n=6	Rumen plus Pepsin plus intestine n=24
B14	B	40.7a	42.3a	79.1a
CM16.5	CM	37.0a	42.8a	83.3b
SBM16.5	SBM	42.8a	43.6a	89.9c
MBM16.5	MBM	24.7b	28.4b	82.5b
CM19	CM	37.5a	41.7a	81.9b
SBM19	SBM	35.0a	35.2ab	92.9c
SEM		2.1	2.4	0.4

[†]Samples were incubated in the rumen for 15 h, placed in pepsin-HCL for 3 h and subsequently inserted into the intestine through duodenal cannulae.

[‡]Diets consisted of a 50:50 concentrate forage (brome/alfalfa hay) mix; Concentrate mixes were B14, barley 14% crude protein (CP); CM16.5, barley/canola 16.5% CP; SBM16.5, barley/soybean meal 16.5% CP; CM19, barley/canola 19% CP; SBM19, barley/soybean meal 19% CP.

[d]Barley was incubated in animals fed B14; canola meal in CM16.5 and CM19; soybean meal in SBM16.5 and SBM19 and meat and bone meal in MBM16.5.

a,b,c Means in the same column with different letters are significantly different ($P<0.05$).

Table IV.3 Ruminal and intestinal crude protein disappearance (%) of total diet (TD) samples using the mobile nylon bag technique.†

Diet Fed‡	Incubated Sample	Rumen n=6	Rumen plus Pepsin HCL n=6	Rumen plus Pepsin plus intestine n=24
B14	B14	33.5	42.2	65.6a
CM16.5	CM16.5	32.3	39.1	70.8b
SBM16.5	SBM16.5	36.8	43.2	70.0b
MBM16.5	MBM16.5	30.7	38.9	71.4b
CM19	CM19	33.3	33.8	73.6b
SBM19	SBM19	36.0	36.9	77.0c
SEM		2.1	2.6	0.4

†Samples were incubated in the rumen for 15 h, placed in pepsin-HCl for 3 h and subsequently inserted into the intestine through duodenal cannulae.

‡ Diets consisted of a 50:50 concentrate forage (brome/alfalfa hay) mix; Concentrate mixes were B14, barley 14% crude protein (CP); CM16.5, barley/canola 16.5% CP; SBM16.5, barley/soybean meal 16.5% CP; CM19, barley/canola 19% CP; SBM19, barley/soybean meal 19% CP.

a,b,c Means in the same column with different letters are significantly different ($P<0.05$).

Table IV.4 Ruminal and intestinal dry matter disappearance (%) of protein sources (PS) using the mobile nylon bag technique.[†]

Diet Fed[‡]	Incubated Sample[d]	Rumen n=6	Rumen plus Pepsin HCl n=6	Rumen plus Pepsin plus Intestine n=24
B14	B	54.5a	56.1a	78.4b
CM16.5	CM	38.1b	44.7b	64.2c
SBM16.5	SBM	40.7b	42.8b	79.9b
MBM16.5	MBM	24.4c	26.3c	57.0e
CM19	CM	40.4b	41.9b	62.5d
SBM19	SBM	48.7a	50.2ab	83.3a
SEM		1.99	2.49	0.51

[†]Samples were incubated in the rumen for 15 h, placed in pepsin-HCl for 3 h and subsequently inserted in the intestine through duodenal cannulae.

[‡] Diets consisted of a 50:50 concentrate forage (brome/alfalfa hay) mix; Concentrate mixes were B14, barley 14% crude protein (CP); CM16.5, barley/canola 16.5% CP; SBM16.5, barley/soybean meal 16.5% CP; CM19, barley/canola 19% CP; SBM19, barley/soybean meal 19% CP.

[d]Barley was incubated in animals fed B14; canola meal in CM16.5 and CM19; soybean meal in SBM16.5 and SBM19 and meat and bone meal in MBM16.5

a,b,c,d,e Means in the same column with different letters are significantly different ($P<0.05$).

Table IV.5 Ruminal and intestinal dry matter disappearance (%) of total diets (TD) using the mobile nylon bag technique.†

Diet Fed‡	Incubated Sample	Rumen n=6	Rumen plus Pepsin HCL n=6	Rumen plus Pepsin plus Intestine n=24
B14	B	32.7	36.5a	54.4a
CM16.5	CM	38.1	38.6ab	51.6b
SBM16.5	SBM	41.0	46.2b	52.3b
MBM16.5	MBM	36.2	42.2ab	51.4b
CM19	CM	37.0	39.4ab	53.2ab
SBM19	SBM	39.4	43.7ab	56.6c
SEM		1.95	2.05	0.51

†Samples were incubated in the rumen for 15 h, placed in pepsin-HCl for 3 h and subsequently inserted in the intestine through duodenal cannulae.

‡ Diets consisted of a 50:50 concentrate forage (brome/alfalfa hay) mix; Concentrate mixes were B14, barley 14% crude protein (CP); CM16.5, barley/canola 16.5% CP; SBM16.5, barley/soybean meal 16.5% CP; CM19, barley/canola 19% CP; SBM19, barley/soybean meal 19% CP.

a,b,c Means in the same column with different letters are significantly different ($P<0.05$).

Table IV.6 Comparison of crude protein (CP) and dry matter (DM) digestibility estimates for total diet (TD) obtained using the mobile nylon bag technique,† and the total fecal collection method.

Diet Fed‡	Incubated Sample	CP		DM	
		Mobile nylon bag	Total fecal collection	Mobile nylon bag	Total fecal collection
B14	B14	65.6	67.2	54.4	68.1
CM16.5	CM16.5	70.8	73.0	51.6	65.7
SBM16.5	SBM16.5	70.0	72.9	52.3	64.9
MBM16.5	MBM16.5	71.7	72.9	51.4	64.3
CM19	CM19	73.6	75.8	53.2	64.9
SBM19	SBM19	77.0	78.4	56.6	68.2

†Samples were incubated in the rumen for 15 h, placed in pepsin-HCl for 3 h and subsequently inserted in the intestine through duodenal cannulae.

‡ Diets consisted of a 50:50 concentrate forage (Brome/Alfalfa hay) mix; Concentrate mixes were B14, barley 14% crude protein (CP); CM16.5, barley/canola 16.5% CP; SBM16.5, barley/soybean meal 16.5% CP; CM19, barley/canola 19% CP; SBM19, barley/soybean meal 19% CP.

D. BIBLIOGRAPHY

AGRICULTURAL RESEARCH COUNCIL. 1985. The nutrient requirements of ruminant livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Farnham Royal, England.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. (AOAC). 1980. Official methods of analysis. 13th ed. Washington, D.C.

CHERIAN, G. 1985. Factors affecting the protein digestibility in studies with the mobile nylon bag technique. M.Sc. thesis, University of Alberta, Edmonton, Alberta.

CHURCH, D.C. 1976. Digestive physiology and nutrition of animals. Vol. 1. O and B. Books, Oregon, USA, 350 pg.

DE BOER, G., MURPHY, J.J., KENNELLY, J.J. and KIRKPATRICK, B.K. 1986. The mobile nylon bag for estimation of intestinal availability of rumen undegradable protein. J. Dairy Sci. (Suppl.) 69:196.

DE BOER, G., MURPHY, J.J. and KENNELLY, J.J. 1987 Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977-982.

HVELPLUND, T. 1985. Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep by an in sacco procedure. Acta Agric. Scand. (Suppl.) 25:

KIRKPATRICK, B.K. and KENNELLY, J.J. 1984. Prediction of digestibility in cattle using a modified nylon bag technique. Can. J. Anim. Sci. 64: 1104.

KIRKPATRICK, B.K. and KENNELLY, J.J. 1987. In situ degradability of protein and dry matter from single protein sources and from a total diet. J. Anim. Sci. 557-567.

NATIONAL RESEARCH COUNCIL. 1985. Ruminant nitrogen usage. National Academy Press, Washington, D.C., U.S.A.

ROOKE, J.A., SEYMOUR, S.J. and ARMSTRONG, D.G. 1981. The effect upon the nutritional value of soyabean meal of incubation in the bovine rumen. Proc. Nutr. Soc. 40: 82A.

SAUER, W.C., JORGENSEN, H. and BERZINS, R. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can. J. Anim. Sci. 63: 233-237.

SETALA, J. 1983. The nylon bag technique in the determination of ruminal feed protein degradation. J. Sci. Agr. Soc. Finland 55: 1.

STEELE, R.G.D. and TORRIE, J.A. 1980. Principles and procedures of statistics. A biometrical approach. 2nd Ed. McGraw-Hill Book Co. Inc., Toronto, Ontario.

VOIGHT, J., PIEATOWSKI, B., ENGELMANN, H. and RUDOLPH, E. 1985. Measurement of the postruminal digestibility of crude protein by the bag technique in cows. Arch. Tierernah., Berlin. 35, 8: 555-562.

V. DYSPROSIUM AS A DIGESTIBILITY MARKER FOR CATTLE [1].

A. INTRODUCTION

The availability of a valid indicator for estimating total tract digestibility would greatly facilitate dairy cattle nutritional research especially with lactating dairy cattle in confinement and loose housing conditions where total collection of feces is difficult or not feasible. Total fecal collection techniques (TFC) are time consuming and labour intensive. Because confinement of animals to metabolic crates is necessary, this technique is not practical under normal dairy cattle management conditions. With dairy cows the additional complication of urine diversion has to be overcome.

Conventional internal indicators such as lignin and acid insoluble ash are variable and are not completely recovered (Van Soest 1982). Chromic oxide (Cr_2O_3) a commonly used external digestibility marker, is subject to diurnal variation and behaves as a heavy liquid passing more quickly from the rumen than coarse fibre particles (Van Soest 1982).

Extensive use of radioisotopes for the evaluation of digestive processes in cattle has been made in recent years. Although radioisotopes have high recovery and low variation they do require the complete collection and disposal of orts, feces and animals. These limitations, tend to prohibite their use in dairy cattle.

Rare earth elements have a high affinity for feed particles (Kennelly et al. 1981b), and further appear to be unabsorbed in the digestion process (Ellis 1968; Young et al. 1976; Kennelly and Aherne 1980). Fecal recovery of rare earth markers in ruminants is usually close to 100% (Young et al. 1976). In swine less variation is observed in estimates of diet digestibility using rare earth elements than with other markers such as polyethyleneglycol and Cr_2O_3 (Kennelly and Aherne 1981).

The instrumental neutron activation analysis (INAA) technique involves the addition of the stable rare earth isotope to the feed material, the concentration of marker in feed and

[1]This chapter has been submitted for publication as a note in the Can. J. Anim. Sci.

fecal samples is then determined by INAA. Although dysprosium (Dy) may be analyzed by techniques such as mass spectrometry, x-ray fluorescence or atomic absorption, these procedures require higher marker concentration, significant sample preparation and are considerably more time consuming than INAA. Dysprosium is extremely sensitive to INAA and can be quantitatively detected at concentrations of 5 ppm.

The objective of this study was to evaluate the efficacy of Dy as an inert marker for the determination of digestibility coefficients in cattle.

B. MATERIALS AND METHODS

Six pregnant Holstein Freisian heifers fitted with permanent rumen and duodenal cannulae were assigned in a 6 x 6 latin square to six dietary treatments. Experimental diets were based on chopped brome alfalfa hay and barley (control) supplemented with canola meal (CM) soybean meal (SBM) or meat and bone meal (MBM). Diets were designed to compare these four protein sources in addition to CM and SBM at two dietary crude protein (CP) levels. The six concentrate mixtures were: barley 14% CP (B14), barley/CM 16.5% CP (CM16.5), barley/SBM 16.5% CP (SBM16.5), barley/MBM 16.5% CP (MBM16.5), barley/CM 19% CP (CM19) and barley/SBM 19% CP (SBM19). Heifers were fed a 50:50 hay concentrate mix in 12 equal amounts each day at 2 h intervals.

Dysprosium ($DyCl_3.6H_2O$) solutions were prepared according to Kennelly et al. (1980). The solution was then sprayed onto 5 kg of ground (2 mm screen) feed (2.5 kg concentrate, 2.5 kg hay) using a common household plant spray bottle. The marker feed mix was dried at 60°C and mixed. A 60 g aliquot was evenly distributed across 12 daily feedings, such that each animal received 36 ppm Dy per d.

Animals were confined in metabolic crates during a 7-d adjustment, 7-d total fecal collection and 3-d digesta collection periods with a minimum of a 7-d rest period, in bedded pens, between each latin square period.

Fecal samples were collected once daily in plastic pans, weighed and an aliquant of total feces was dried in a forced-air oven at 65°C for 48 h. In order to evaluate diurnal variation additional grab samples for each diet were collected during one period and processed similarly to the total fecal collection samles. Upon completion of each period, total fecal samples were pooled on a per animal basis and stored for later dry matter (DM), nitrogen (N) and Dy analysis. Kjeldahl N analysis was as outlined by the Association of Official Analytical Chemists (1980, AOAC) (method no. 7015) .

Dysprosium content of feed and feces were determined by INAA as outlined by Kennelly et al. (1980). Recovery of Dy was determined and digestibility coefficients were then calculated using Dy ratio in feed and feces.

All data were analysed using analysis of variance procedures with treatment, animal and period as factors. Treatment means were compared by the Student-Newman-Keuls test when treatment effect was significant (Steele and Torrie 1980).

C. RESULTS AND DISCUSSION

Dry matter digestibility (DMD) coefficients predicted by the Dy ratio technique are slightly lower, but highly correlated (r=.86) with the TFC method (Table V.1). These results are in good agreement with results of previous research with cattle. Olbrich et al. (1971) using cerium (Ce), and Young et al. (1976) using Dy, reported DMD by the Dy ratio technique versus the TFC technique to be 55.9 vs 59.8 and 66.1 vs 68.7, respectively. Kennelly et al. (1980) found the Dy ratio technique to be an accurate predictor of digestibility coefficients in pigs. Crude protein digestibility coefficients are slightly overestimated by the Dy ratio technique when compared with TFC (Table V.1) (r=.79). These results (Table V.1) are in good agreement with Olbrich et al. (1971) using Ce in bulls and Kennelly et al. (1980) using Dy in growing pigs.

An important characteristic of an external digestibility marker is that it must bind tightly to ingested feed particles and remain bound throughout the digestive process. Research

by Hartnell and Satter (1979) suggests that the rare earth elements samarium (Sa) and lanthenum (La) remain tightly bound through the digestive tract. However, Kennelly et al. (1981a,b) with Dy (incubated in rumen fluid (pH 6.4)) found that high proportions of the Dy did not remain bound to the particulate fraction but precepitated out or could be found in the supernatant fraction. Further, Combs et al. (1984) with ytterbium (Yb) and Ce determined that at low pH (in the abomasum) these rare earth elements, particularly Yb, do not remain with the digesta particles but migrate into the liquid phase. Further, it has been suggested by Miller et al. (1967) that lower recovery rates of Ce in cattle might be due to adsorption of the rare earth on to the lining of the digestive tract.

A second characteristic of a digestibility marker is that it must not be digested or incorporated into body tissues. Kennelly et al. (1980) with pigs and Koyama and Miyamoto (1985) with goats found no traces of Dy in urine. Koyama and Muyamoto (1985) found no rare earth residues in several organs and tissues of goats fed Europium (Eu) in long term studies. It should be noted, however, that the lining of the small intestine was not examined for residues. The percent recovery of Dy averaged 95.4% (Table V.1) for the six diets examined. This is in agreement with Young et al. (1976) and Koyama and Miyamoto (1985) who found 91.3% recovery in steers and 95.6% recovery in goats. respectively. Siddons et al. (1985) using Yb found 103% recovery, while Ellis (1968) using Ce found 99.8% recovery. Kennelly et al. (1980) demonstrated 100% recovery of Dy fed to pigs. Variation in recovery may be due, in part, to marker administration. Young et al. (1976) found lower recovery in steers when Dy was administered twice daily in the form of a bolus. Lower recovery has also been attributed to adsorption of the rare earth on surface of the digestive tract (Miller et al. 1967).

The DM and CP digestibility values obtained for the four subsamples collected over a 24 h period (for one cow on each diet) are in good agreement with the corresponding TFC results (Table V.2). Ellis (1968) and Olbrich et al. (1971) evaluated within day variation of fecal Dy concentration for animals fed twice daily and found it to be minimal. The absence of

significant within day variation indicates that DM and CP digestibility can be reliably determined by daily grab sampling. Further, Kennelly et al. (1980), Olbrich et al. (1971) and Ellis (1968) found day to day variation to be minimal.

Use of rare earth elements, such as Dy in digestibility studies with dairy cattle eliminates the requirement for total collection of feces and allows digestibility coefficients to be estimated by grab sampling. The technique can be used to obtain reliable estimates of coefficients in situations where the use of conventional methods for measuring digestibility are impractical.

Table V.1 Intake and Digestion of Dry Matter (DM) and crude protein (CP) as Measured by Total Fecal Collection (TFC) or Dysprosium (Dy) and percent recovery of Dy.

	Diet and Protein Source						
	B14	CM16.5	SBM16.5	MBM16.5	CM19	SBM19	SE
DM Intake (kg/day)	8.55	8.55	8.55	8.55	8.55	8.55	
DM Digested%							
TFC	68.1	65.1	64.6	64.3	64.9	68.2	0.96
Dy	65.1a	63.0ab	62.5ab	59.4b	61.4ab	64.6a	1.16
CP Intake (kg/day)	1.16	1.48	1.53	1.50	1.71	1.76	
CP Digested%							
TFC	67.2a	73.0b	72.9b	72.9b	75.8bc	78.4c	1.10
Dy	69.8	76.4	75.4	76.2	73.7	77.8	0.84
Dy recovery %	95.6	96.8	96.7	92.0	94.6	94.7	

a,b,c, Means in the same row with different letters are significantly different ($P<0.05$).

Table V.2 Variation in digestibility coefficients as determined using the dysprosium ratio technique on four fecal grab samples collected over a 24 hr period.

Diet	Subsample number and Digestibility Coefficients				
	1	2	3	4	TFC†
Dry matter digestibility coefficients					
B14	65.9	66.0	63.6	65.6	68.1
CM16.5	62.3	62.5	63.0	59.8	65.1
SBM16.5	59.5	61.6	60.7	59.6	64.6
MBM16.5	58.1	59.2	59.6	N/A	N/A
CM19	N/A	N/A	N/A	N/A	64.9
SBM19	64.6	64.5	63.7	66.0	68.2
Crude protein digestibility coefficient					
B14	71.8	72.3	69.9	70.2	67.2
CM16.5	79.0	78.1	79.9	76.7	73.0
SBM16.5	76.6	72.0	72.6	70.1	72.9
MBM16.5	74.6	75.0	76.0	N/A	72.9
CM19	N/A	N/A	N/A	N/A	75.8
SBM19	79.0	78.1	77.6	80.7	78.4

†Digestibility coefficients determined using total fecal collection techniques

D. BIBLIOGRAPHY

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1980. Official method of analysis. 12th ed. AOAC, Washington, D.C.

COMBS, D.K., SHAVER, R.D., SINGH, N. and SATTER, L.D. 1984. Effects of application procedure, pH and microbial growth on retention of ytterbium and cerium by brome hay. Can. J. Anim. Sci. 64. (Suppl. 1). 66-67.

ELLIS, W.C. 1968. Dysprosium as an indigestible marker and its determination by radioactivation analysis. J. Agr. Food Chem. 16: 220-224.

HARTNELL, G.F. and SATTER, L.D. 1979. Extent of particulate marker (samarium, lanthanum and cerium) movement from one digesta particle to another. J. Anim. Sci. 48: 375-380.

KENNELLY, J.J., APPS, M.J., TURNER, B.V. and AHERNE, F.X. 1980. Dysprosium, cerium and chromium marker determination by instrumental neutron activation analysis. Can. J. Anim. Sci. 60: 749-756.

KENNELLY, J.J. and AHERNE, F.X. 1980. Dysprosium as a marker for routine feed evaluation. Can. J. Anim. Sci. 60: 1052-1057.

KENNELLY, J.J. and AHERNE, F.X. 1981. Evaluation of chromic oxide, acid insoluble ash, dysprosium and polyethylene glycol as markers in swine digestibility studies. J. Anim. Sci. 53 (Suppl.1):492.

KENNELLY, J.J., YANG, M.T. and MILLIGAN, L.P. 1981a. Influence of buffers, pH and rumen fluid on dysprosium precipitations in vitro. J. Anim. Sci. 53 (Suppl. 1):410.

KENNELLY, J.J., YANG, M.T. and MILLIGAN, L.P. 1981b. Attachment of rare earth markers to particulate matter. J. Anim. Sci. 53 (Suppl. 1):492-493.

KOYAMA, T. and MIYAMOTO, S. 1985. Feasibility studies in goats fed rare earth elements as ingestion markers. Jpn. J. Zootech. Sci. 55: 911-916.

MILLER, J. K., PERRY, S.C., CHANDLER, P.T. and CRAGLE, R. G. 1967. Evaluation of radiocerium as a nonabsorbed reference material for determining gastrointestinal sites of nutrient absorption and excretion in cattle. J. Dairy Sci. 50:355-361.

OLBRICH, S.E., MARTZ, F.A., VOGT, J.R. and HILDERBRAND, E.S. 1971. Use of neutron activation analysis in the determination of digestibility with cerium as an inert marker. J. Anim. Sci. 33: 899-902.

SIDDONS, R.C., PARADINE, J., BEEVER, D.E. and CORNELL, P.R. 1985. Ytterbium acetate as a particulater phase digesta-flow marker. Br. J. Nutr. 84: 509-519.

STEELE, R.G.D. and TORRIE, J.W. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Company. New York.

VAN SOEST, P.J. 1982. Nutritional Ecology of the Rumen. O & B Books, Inc. Corvallis, Oregon.

YOUNG, M.C., THEURER, B., OGDEN, P.R., NELSON, G.W. and HALE, W.H. 1976. Dysprosium as an indicator in cattle digestion trials. J. Anim. Sci. 58: 1270-1279.

VI. GENERAL DISCUSSION

The objectives of the research described herein were to evaluate the nutritional quality of protein sources in the diets of pregnant heifers. The protein sources and their respective total diets were examined in terms of their ruminal degradability, protein supply to and availability in the small intestine. In addition, the efficacy of dysprosium as a digestibility marker for ruminants was examined.

Protein feeding systems should be able to predict the proportion of feed nitrogen (N) which will become available to the rumen microbes (RDP) and the subsequent proportion which is available for digestion in the small intestine (UDP). These systems therefore will require information on the chemical composition of feeds plus estimates of digestion by rumen microbes and intestinal disappearance and subsequent tissue utilization. These concepts have been recognized in the development of several new protein feeding systems which have been proposed over the past ten years. The difficulty arises in finding techniques which accurately measure feed protein degradability in the rumen and digestibility in the intestine. It is generally agreed that in vivo techniques are most accurate even though they have the greatest technical complexity. The in situ nylon bag technique is widely used in the determination of rumen degradability because it is relatively rapid and correlates well with in vivo results (Madsen and Hvelplund 1985; Oldham 1983). The in situ technique (Ørskov and McDonald 1979) assumes that the measurement of protein residue remaining in the bag at any one point in time can provide an estimate of protein degradation. The technique estimates the size of three protein fractions: soluble, ruminally degradable and ruminally undegradable.

The first experiment (Chapter II) reported herein used the in situ nylon bag technique to measure RDP and UDP of several protein sources (canola meal (CM), meat and bone meal (MBM), soybean meal (SBM) and barley (B)) and two protein levels (for CM and SBM). The individual proteins were evaluated as well as the total diets in which they were incorporated. Data from the in situ nylon bag technique are combined with estimates of ruminal outflow rates to determine effective degradability.

Several factors may affect the estimates of effective protein degradability (EDP). These include the presence of the protein source being tested in the basal diet; (Loerch et al. 1983) (Vik-Mo and Lindberg 1985) fractional outflow rate (Amaning-Kwarleng et al. 1986); physiological state (Gonzalez et al. 1985) and concentrate to forage ratio (Barrio et al. 1985). For these reasons and because of the difficulty in obtaining in vivo data the in situ technique should be used primarily to rank protein sources and diets (on the basis of RDP and UDP values) relative to one another under specifically described feeding and physiological conditions. The EDP values for individual protein sources obtained in this experiment (using a fractional outflow rate of .05 h^{-1}) tended to increase with increasing level of protein in the diet, however, this result was not consistent across protein sources or rumen incubation times. The EDP values indicated that the MBM product was most, SBM intermediate and CM least resistant to rumen microbial attack. The low EDP value for MBM suggests that, if properly processed, MBM may be an effective source of UDP for dairy cattle.

The second experiment (Chapter III) used the in-vivo technique to estimate RDP and UDP for the various protein sources and diets. In vivo measurements of UDP flow to the small intestine are assumed to be pivotal in the evaluation of ruminal protein degradation. Validation of results from the in situ technique are dependent upon the correlation of in vivo and in situ results, however, the in vivo measurements are subject to several potential sources of error. Estimates of microbial N reaching the small intestine vary depending on the technique employed, the animal and the diet (Arambel 1987). Errors in estimates of solid and liquid flow rate to the small intestine can result in significant variation in estimates of protein reaching the small intestine (ARC 1985), therefore, choice of marker and method of analysis can be critical. Madsen and Hvelplund (1985), compared in vivo and in situ data for a wide variety of feeds fed to dairy cows. They found a good relationship between the two methods when a fractional outflow rate of .08 h^{-1} was assumed. They recommended standardized diets for experimental animals and that animals be fed small amounts of a wide variety of protein supplements.

In general, dry matter (DM) digestion and flow to the small intestine were not affected by protein source or concentration. Ruminal organic matter (OM) digestion was affected ($P<.05$) by protein source; MBM being lower than all other diets. Total N flow to the small intestine for the three diets formulated on a UDP equivalent basis was 224.0, 225.6 and 241.1 g N/day for MBM, CM and SBM, respectively. Microbial N flow to the duodenum was not affected by protein source or concentration. Dietary UDP concentrations were not affected by protein source, however, the MBM16.5 diet tended to be less degradable than the barley or CM amd SBM diets at the lower level of inclusion.

A comparison based on predictive (ARC 1985; NRC 1985) estimates and actual calculation of dietary RDP and UDP are presented in Chapter III (Table III.6). In situ degradability estimates are influenced by dietary protein source and energy level (Loerch et al. 1983; Madsen and Hvelplund 1985), and rumen pH (Loerch et al. 1983). Madsen and Hvelplund (1985) found the differences in degradability estimates were confounded for vegetable protein sources but not for fish meal protein sources. Comparison of in situ versus in vivo estimates for UDP indicate that the in vivo technique results in a higher estimate for vegetable protein diets and a lower estimate for the MBM diet. Theoretically, the new protein feeding systems provide a more accurate prediction of the protein supply to the small intestine, and thus improved prediction of animal performance. Results of the above series of experiments suggest that animal performance can be more accurately predicted when diets are formulated on the basis of RDP and UDP, than when diets based on crude protein (CP) or digestible crude protein.

Under normal feeding conditions the amount of UDP entering the small intestine is less than the protein contributed by rumen microorganisms. However, the amino acids supplied (quantitatively and qualitatively) by the UDP fraction will have a significant impact on the value of the feed protein, especially in animals at high levels of production.

The mobile nylon bag technique (MNBT) as a method for determining ruminal and intestinal CP digestibility was evaluated in the third experiment (Chapter IV).

Hvelplund (1985), using sheep, estimated true digestibilities of SBM and CM to be 97% and 76%, respectively. The low CM digestibility was attributed to high levels of cell wall bound nitrogen. Voight et al. (1985) determined the true digestibility of SBM, CM and B in dairy cattle to be 97.2, 91.8 and 89.6%, respectively. De Boer et al. (1986) estimated the true digestibility of MBM CP to be 80.1% after 24 h incubation in the rumen. The results of the present study for CP digestibilities are in good agreement with the above research. A comparison between the MNB technique and the traditional total fecal collection method for the determination of total tract CP digestibility resulted in a high correlation ($r = .99$).

The availability of a valid indicator for estimating total tract digestibility would greatly facilitate dairy cattle nutritional research, especially with lactating dairy cattle in confinement and loose housing conditions where total collection of feces is difficult or infeasible. The fourth experiment Chapter IV, was conducted to evaluate the efficacy of dysprosium (Dy) as an inert external marker for the determination of digestibility coefficients in dairy cattle. The use of instrumental neutron activation analysis for the measurement of Dy in feed and feces has been established (Kennelly and Aherne 1980, 1981). The results of the present experiment demonstrated that Dy can be used as a digestibility marker in dairy cattle. The procedure eliminates the requirement for total collection of feces and allows digestibility coefficients to be estimated by grab sampling of feces. The technique results in reliable estimates of digestibility coefficients in situations where the use of conventional methods for measuring digestibility are impractical.

A. BIBLIOGRAPHY

AGRICULTURAL RESEARCH COUNCIL (ARC). 1985. The Nutrient Requirements of Ruminant livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Farnham Royal, England.

AMANING-KWARLENG, K., KELLAWAY, R.C. and LIEBHOLZ, J. 1986. Rumen degradation and fractional outflow rates of nitrogen supplements given to cattle eating sodium hydroxide treated straw. Br. J. Nutr. 55: 387-398.

ARAMBEL, M.J., BARTLEY, E.E., DUFVA, G.S., NAGARAJA, T.G. and DAYTON, A.D. 1982. Effect of diet on amino and nucleic acids of mixed rumen bacteria and protozoa. J. Dairy Sci. 65, 2095-2101.

BARRIO, J.R., OWENS, F.N. and GOETSCH, A.L. 1985. Soluble nutrients in protein supplements and in situ disappearance. Can. J. Anim. Sci. 65: 667.

DE BOER, G., MURPHY, J.J. and KENNELLY, J.J. 1987 Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977-982.

GONZALEZ, J.S., ROBINSON, J. and FRASER, C. 1985. The effect of physiological state on digestion in the ewe and its influence on the quantity of protein reaching the abomasum. Livestock Prod. Sci. 12: 59.

HVELPLUND, T. 1985. Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep by an in sacco procedure. Acta Agric. Scand. (Suppl.) 25:

KENNELLY, J.J. and AHERNE, F.X. 1980. Dysprosium as a marker for routine feed evaluation. Can. J. Anim. Sci. 60: 1052-1057.

KENNELLY, J.J. and AHERNE, F.X. 1981. Evaluation of chromic oxide, acid insoluble ash, dysprosium and polyethylene glycol as markers in swine digestibility studies. J. Anim. Sci. 53 (Suppl. 1): 492.

LOERCH, S.C., L.L. BERGER, D. GIANOLA and G.C. FAHEY, JR. 1983. Effects of dietary protein source and energy level on in situ nitrogen disappearance of various protein sources. J. Anim. Sci. 56:206.

MADSEN, J. and T. HVELPLUND. 1985. Protein degradation in the rumen. A comparison between in vivo, nylon bag, in vitro and buffer measurements. J. Acta Agric. Scand. 25:103.

NATIONAL RESEARCH COUNCIL. 1985. Ruminant nitrogen usage. National Academy Press, Washington, D.C., U.S.A.

OLDHAM, J.D. 1983. New systems for assessing protein needs for ruminants. Alberta Dairy Seminar, Banff, Alberta.

ØRSKOV, E.R. and MCDONALD, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. Camb. 92: 499-503.

VIK-MO, L. and J.E. LINDBERG. 1985. In sacco degradability of protein (N) and dry matter (DM) in samples of individual feeds or combinations; tested with diets medium or high in protein. Acta Agr. Scand. 35:117.

VOIGHT, J., PIEATOWSKI, B., ENGELMANN, H. and RUDOLPH, E. 1985. Measurement of the postruminal digestibility of crude protein by the bag technique in cows. Arch. Tierernah., Berlin. 35, 8: 555-562.